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# THE EFFECTS OF OVARIAN HORMONES AND SEASONS ON ANOLIS CAROLINENSIS

## I. THE THYROID

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ONE PLATE (TEN FIGURES)

### INTRODUCTION

The following workers have reported on thyroid relationships in reptiles. Viguier ('11) mentioned by Siler ('36) found marked changes in the pituitary of the lizard, Uromastix acanthinurus, as a result of thyroidectomy. Thyroidectomy delays moulting in Lacerta agilis (Drzewicki, '28) and Hemidactylus brookii (Noble and Bradley, '33). The latter also report that hypophysectomy delays moulting. However, thyroidectomy or hypophysectomy actually hasten the periods of skin shedding in the garter snakes, Thamnophis sirtalis and Thamnophis radix (Schaefer, '33). At 70° F. thyroidectomy causes marked degeneration of basophilic cells of the anterior pituitary in the garter snake, although at 90° F. much less degeneration is apparent (Siler, '36). Hellbaum ('36) describes atrophy induced in the thyroid of the garter snake by hypophysectomy, and hypertrophy by activation with anterior pituitary injections.

The purpose of this study is to discover, if possible, a structural or physiological explanation for certain observed changes in mating and territorial behavior of the species following castration (Evans, '36 a, b, c; '37 a, b).

The senior author wishes to thank Dr. A. B. Dawson of Harvard for his kindly interest in the general problem of the

effect of theelin upon the lizard, of which this paper forms a part. The assistance of Dr. F. L. Hisaw of Harvard is also gratefully acknowledged. Photographs were prepared by George Christensen, Montana State University.

## MATERIALS

The present problem was divided into two parts. The first of these was carried out during the winter of 1935 when each of nine young males (average weight 1.98 gm.) and nine young females (average weight 1.77 gm.) received an average dose of 9 r.u. of aqueous theelin,[1] at a rate of 1 r.u. every other day. Nine young males (average weight 2.77 gm.) and thirteen young females (average 1.76 gm.) served as controls. All of these lizards were killed between November 27 and December 11, 1935.

The second part was conducted during the late spring of 1936 and involved a study of five spayed females (ovariectomized 14 weeks prior to June 12), eight normal females, and nine normal males, all of which were killed on June 12, 1936.

## METHODS

Tissues were fixed in either Helly's (7½% formalin instead of the usual acetic acid) or in Bouin's, cut at 5 μ and stained in Harris' modification of Delafield's haematoxylin. No attempt was made to weigh the very minute thyroids.

The follicles were carefully measured and the average mean diameters for each animal are recorded in table 1. The average height of at least sixty individual cells of representative large, medium and small sized follicles of each animal were recorded. All measurements were obtained with an ocular micrometer which was carefully checked for accuracy with a stage micrometer. The animals were fed and otherwise cared for adequately as described elsewhere (Evans, '36 c).

---

[1] Kindly supplied by Dr. Oliver Kamm of Parke, Davis Company.

## RESULTS

Table 1 gives the results of the winter experiments. It will be noted that the thyroids of the males responded to subcutaneous theelin injections more markedly than did those of the females. The follicles of male controls showed an average mean diameter of 53.78 μ, or 38% larger in diameter than the follicles of the theelin injected males, which averaged 33.38 μ.

TABLE 1

*Effects of theelin on thyroids of young Anolis carolinensis; winter*

| CONTROLS | | | | THEELIN INJECTED | | | | |
|---|---|---|---|---|---|---|---|---|
| Name | Weight, gm. | Average diameter follicles in μ | Average height cells in μ | Name | Weight, gm. | r.u. theelin | Average diameter follicles in μ | Average height cells in μ |
| | | | | Males | | | | |
| C6 | 3.6 | 56.52 | 4.5 | D7 | 3.2 | 11.5 | 36.55 | 5.7 |
| C9 | 3.3 | 52.70 | 3.9 | D9 | 3.2 | 4.5 | 29.32 | 4.6 |
| C8 | 3.3 | 63.75 | 4.1 | D4 | 2.5 | 9.2 | 38.25 | 5.7 |
| C7 | 3.2 | 48.02 | 4.4 | D1 | 2.2 | 5.5 | 38.25 | 5.6 |
| C3 | 2.9 | 43.35 | 4.2 | D6 | 2.1 | 11.0 | 27.62 | 5.7 |
| C5 | 2.5 | 69.70 | 4.3 | D8 | 1.5 | 4.5 | 18.70 | 5.3 |
| C4 | 2.4 | 52.27 | 4.6 | D3 | 1.2 | 11.5 | 30.60 | 5.3 |
| C1 | 2.1 | 45.90 | 6.8 | D5 | 1.0 | 11.5 | 33.15 | 4.9 |
| C2 | 1.7 | 51.85 | 5.1 | D10 | 1.0 | 11.5 | 48.02 | 5.8 |
| Av. | 2.77 | 53.78 | 4.65[1] | | 1.98 | 8.9 | 33.38 | 5.4 |
| | | | | Females | | | | |
| B1 | 2.50 | 48.87 | 5.40 | E1 | 2.7 | 11.0 | 47.60 | 5.40 |
| B4 | 2.50 | 51.81 | 4.95 | E8 | 2.5 | 4.5 | 55.25 | 5.43 |
| B8 | 2.40 | 72.67 | 4.95 | E10 | 2.5 | 11.0 | 39.95 | 4.69 |
| B2 | 2.30 | 76.50 | 5.21 | E2 | 1.8 | 11.0 | 79.47 | 5.47 |
| B6 | 2.09 | 61.41 | 4.27 | E3 | 1.5 | 11.0 | 33.15 | 4.55 |
| B11 | 1.85 | 70.55 | 5.19 | E9 | 1.5 | 11.0 | 30.17 | 4.44 |
| B7 | 1.80 | 50.49 | 4.95 | E12 | 1.25 | 11.0 | 31.87 | 4.66 |
| B3 | 1.70 | 39.95 | 4.77 | E13 | 1.15 | 11.0 | 42.50 | 4.40 |
| A4 | 1.30 | 37.35 | 3.99 | E6 | 1.10 | 11.5 | 33.15 | 5.50 |
| A3 | 1.20 | 42.00 | 4.07 | Av. | 1.77 | 10.3 | 43.67 | 4.94 |
| A5 | 1.20 | 54.40 | 4.55 | | | | | |
| B9 | 1.10 | 53.42 | 4.32 | | | | | |
| B10 | 1.00 | 66.72 | 4.33 | | | | | |
| Av. | 1.76 | 55.83 | 4.69 | | | | | |

---

[1] Represents average height of cells of male winter controls. The average is 4.38 μ not counting C1 which seems to be goitrous.

On the other hand, the average height of individual follicular cells of the theelin injected males was 5.4 μ or 14.1% higher than the follicular cells of controls, which averaged 4.65 μ.

The average mean diameter of the follicles of female controls was 55.83 μ or 23% larger than those of the theelin injected females whose average was 43.67 μ. The average height of the cells of the theelin injected females was 4.94 μ or 5.1% higher than those of the control females which averaged 4.69 μ.

It was noted in all of the winter thyroids, but particularly in the male theelin series, that a relatively large number of very small and hyperplastic follicles abounded in the central portions of the glands, larger follicles were farther from the center, while at the periphery a number of large follicles were observed to be in a stage of degeneration as indicated by loss of discrete cells and nuclei and a transformation of the epithelial cells into fibrous connective tissue. These large and apparently aged follicles at the periphery were often ruptured or collapsed and devoïd of colloid. This indicates that the follicles of the thyroid gland are being formed from clusters of potential epithelial cells which lie in the central portion of the gland. As these centrally located follicles enlarge, they are caused to shift more and more toward the surface of the gland because of the more active growth of still younger follicles, until at last when their period of active function is over they are found at the outer periphery of the thyroid in an atrophied and fibrous condition.

Twenty-two lizards killed on June 12, 1936 were distributed as follows: nine young males (average, 3.5 gm.); five females which had been spayed 14 weeks previously (average, 2.5 gm.); eight normal females, three of which were ovulating at the time they were killed (average, 2.3 gm.). Study of these thyroids showed the average mean diameter of the follicles of these lizards to be: normal males, 81.59 μ; spayed females, 69.81 μ: normal non-ovulating females, 63.50 μ; normal ovulating females, 51.0 μ. The average height of the follicular cells of these same lizards was: males, 3.11 μ; spayed females, 3.34 μ; normal non-ovulating females, 3.44 μ; normal ovulating females 7.11 μ. The colloidal contents of the follicles were relatively clear and unvacuolated except for those of the ovulating females. In these the contents were slightly vacuolated.

## DISCUSSION

The evidence brought forth in the preceding paragraphs indicates that theelin stimulates the thyroids of young lizards to a marked degree when studied at room temperature (70°F.) during November and December. This stimulation is shown not only by an increase in the height of individual cells but also by an increase in the number of young follicles in the central region of the thyroid.

In the garter snake (Hellbaum, '36), anterior pituitary injections cause increase in height of follicular cells of the thyroid and the follicles themselves are smaller than those of controls. This response is similar in degree to that obtained in Anolis with theelin.

By reducing the temperature, the central part of the thyroid in the rat (Baillif, '37) can be markedly stimulated while the peripheral portions remain unaffected. In Anolis the winter control thyroids were characterized by relatively smaller follicles with higher epithelial walls than those of late spring control thyroids. But this difference does not seem to be due to change in temperature since the winter Anolis were continuously kept at 70°F. Baillif's work verifies our observations that the central region of the thyroid is the part which responds to stimulation.

## SUMMARY

The average mean diameter of the follicles of eighteen lizards injected with theelin during the winter was markedly smaller than that of twenty-two controls. This suggests that theelin stimulates the formation of young follicles which appear in the central part of the gland. The height of the follicular cells was greater in injected lizards. Theelin was found to have a greater effect upon the winter thyroid of the male than upon the female.

Except for those of three ovulating females, the follicles of twenty-two uninjected lizards of the late spring were much larger than those of winter controls. Those of the ovulating females were slightly smaller than those of the winter female controls, and they were considerably smaller in diameter than those of the late spring series of non-ovulating females, of five spayed females, and of nine normal males. The height of the follicular cells of females ovulating in the late spring was considerably greater than that of any of the other late spring or of the winter series of lizards.

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PLATE

## PLATE 1

### EXPLANATION OF FIGURES

Thyroids of Anolis carolinensis, winter 1935–1936 and later spring 1936. Figures 1 and 3, × 287.6; all other figures, × 480.

Males, winter, controls:

1 C4, weight 2.4 gm. Note relatively large acini as compared with figure 3.
2 C7, weight 3.2 gm.

Males, winter, theelin injected:

3 D6, weight 2.1 gm., 11 r.u.
4 D7, weight 3.2 gm., 11 r.u.

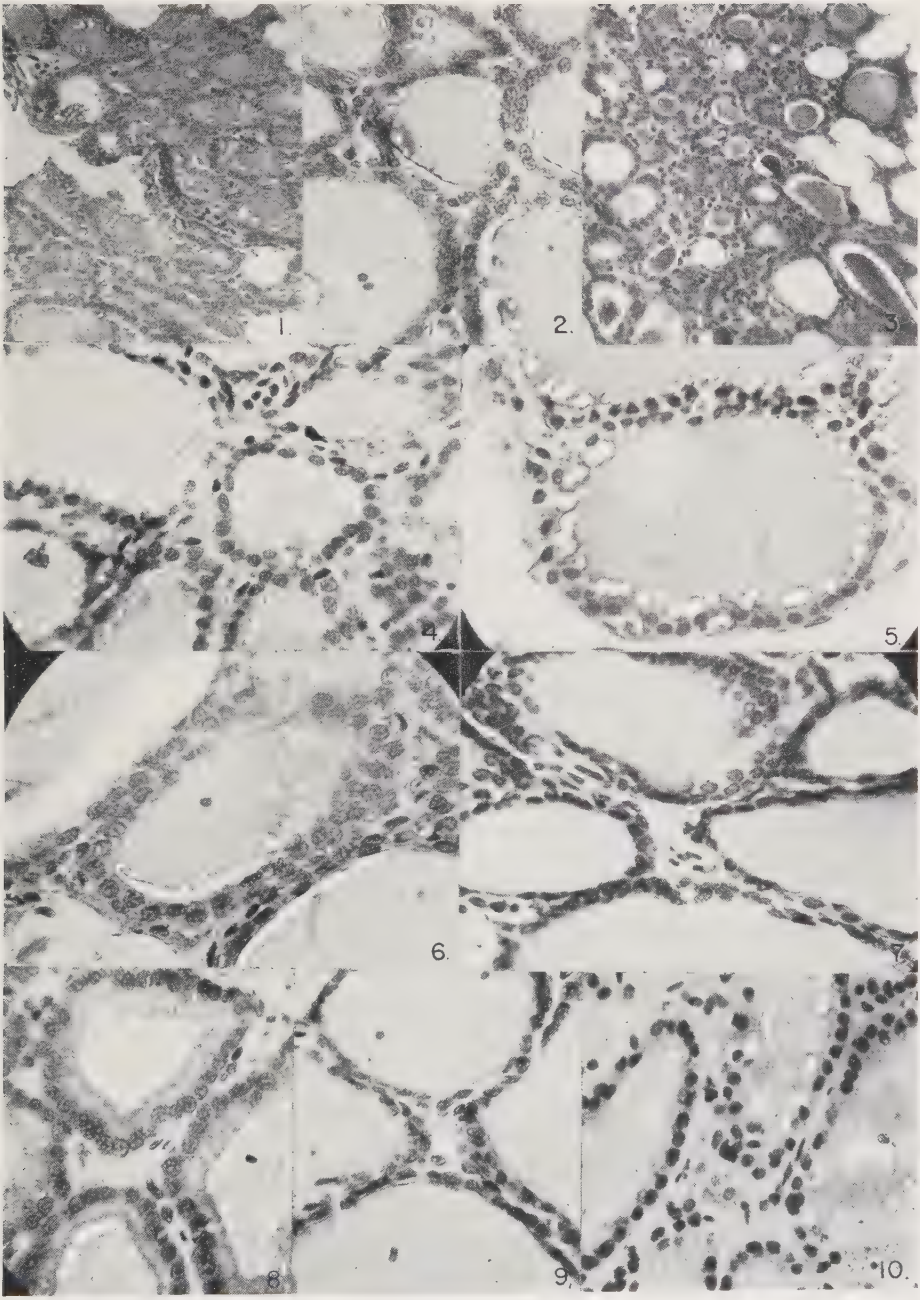
Females, winter:

5 B7, control, weight 1.8 gm.
6 E2, injected, weight 1.8 gm., 11 r.u.

7 Male, control, late spring, weight 3.5 gm.

Females, late spring:

8 Normal, mature ovum in oviduct, weight 2.5 gm.
9 Normal, no mature ova present in ovary or oviducts, weight 2.2 gm.
10 Spayed February 28, 1936, weight 2.5 gm.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# THE OVARIES OF THE BAT MYOTIS LUCIFUGUS LUCIFUGUS AFTER INJECTION OF HYPOPHYSEAL EXTRACT

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SIX FIGURES

## INTRODUCTION

Bats of the genus Myotis, resident in the limestone caves of Missouri during the winter, normally ovulate late in March and in April when they leave the caves. Parturition occurs from the middle to the end of June, and lactation continues through July. By mid-September one follicle, which is the follicle of ovulation, contains a small antrum and females are receptive to males. During the so-called hibernation season, which begins in October, the bats are not continuously dormant, elimination of sperm occurs, and there is indirect evidence of vernal inseminations as the period of ovulation approaches (Guthrie, '33 b).[1] It has been suggested that the females are in a condition of submaximal oestrus throughout the winter (Guthrie and Jeffers, '38 b).

It seemed certain that ovulation of the vesicular follicle in Myotis could be induced readily by injection of hypophyseal extract (compare Zondek, '33; Caffier, '34; Caffier and Kolbow, '34; and Herlant, '34). If the sperm of the autumnal inseminations are responsible for the vernal activation, as is widely held by students of the bat (see summary by Baker and Bird, '36), normal development should ensue if ovulation occurred prior to March or April. An indirect method of attack on the problem of longevity of functional sperm in the bat was available.[2]

---

[1] Miller ('36) has reported that the epididymides of males of M. lucifugus lucifugus are packed with sperm and that the accessory glands are in an active state throughout the hibernating period.

Further, in interpreting the growth of follicles during the period of submaximal oestrus, it had seemed necessary to assume that follicles at all stages in differentiation were responsive to a gonadotropic substance which was available in only limited amount during the winter (Guthrie and Jeffers, '38 b). If a census of all normal growing follicles and those in early stages of retrogression in the ovaries of specimens injected with hypophyseal extract were made for comparison with normal specimens, it should be possible to determine the accuracy of these assumptions.

## MATERIAL AND METHODS

Female bats of the species Myotis sodalis and M. lucifugus lucifugus were given a single intraperitoneal injection of hypophyseal extract containing gonadotropic hormone each day for 3 days and killed at varying intervals after the last injection. We are indebted to Dr. J. B. Collip of McGill University who furnished the extract and stated its assay to be "slightly less than 0.1 cc. per day rat unit."

The relative ages of the bats were judged by the wear on their teeth. Throughout the experiments the animals were kept isolated from males at about 18°C. in cages with moist coverings; they were fed daily.[3] Killing was by decapitation, and the ovaries and reproductive tracts were fixed in Champy's fluid and post-chromed. Dehydration with butyl alcohol was followed by embedding in paraffin, and serial sections of the ovaries were cut at 4 μ. Reproductive tracts were sectioned serially at 5 μ. The sections were stained with acid fuchsin by Kull's method.

---

[2] The apparently obvious method of determining this point by keeping females isolated during the hibernating period has not been feasible. Cave conditions could not be duplicated in our laboratory and females died when caged in the caves, which suggests that lack of water may be the stimulus which awakens bats from their sleep during the winter. That awakening is spontaneous is clear from the observations of flying bats that can be made by any visitor to a hibernation roost in Missouri.

[3] While bats in captivity will eat a considerable variety of food they do not eat enough to keep themselves alive. Consequently all experimental animals were fed daily on a ration obtained through the courtesy of Prof. A. G. Hogan, of the University of Missouri.

Counts and measurements of all the normal growing follicles and of follicles in early stages of degeneration were made for both ovaries of twenty-two injected M. lucifugus lucifugus in the same manner as for a series of normal animals (Guthrie and Jeffers, '38 b). Two diameters of each follicle and its contained oocyte were measured and the average taken as the diameter of the structure. Measurements were made at a magnification of 600 diameters, using an eye-piece micrometer.

## OBSERVATIONS

### *Ovulation and development of ova*

*M. sodalis.* Three young individuals with no wear on their teeth, collected December 4, 1935, were injected with 0.1 cc. of hypophyseal extract on December 9th, 10th and 11th (table 1, nos. 1280, 1281 and 1282). Two of the bats were killed December 12th, the third on December 19th. Ovulation had occurred in each of these specimens. A tubal egg which had been activated but had not divided was recovered from the tract of each of the animals killed December 12th; it appears improbable that normal development would have followed. An egg undergoing fragmentation was found in the uterus of the third bat; whether or not activation had occurred could not be determined.

Three other young individuals collected December 4th remained in the laboratory for 12 days and then were injected with 0.05 cc. of the extract on December 16th, 17th and 18th (table 1, nos. 1, 2 and 1290). In only one animal, dead December 19th, did ovulation occur. The tract of this bat was not fixed and the condition of the egg is not known.

Four other bats, only one of which had any wear on the teeth, collected December 4th, were kept for 15 days before injections with hypophyseal extract were begun. One was killed after two injections of 0.1 cc. each. Ovulation had not occurred in this animal. The other three bats were

TABLE I

*Results of injection of hypophyseal extract in female bats (Myotis)*

| Date collected | December 4, 1935 | | | | | | | | | | January 7, 1936 | | | | | | | | | | February 20, 1936 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Species | M. sodalis | | | | | | M. lucifugus lucifugus | | | | M. lucifugus lucifugus | | | | | | | | | | M. lucifugus lucifugus | | | | | | | | | |
| Number of animal | 1280 | 1281 | 1282 | 1 | 2 | 1290 | 3 | 1283 | 1284 | 1289 | 1307 | 24 | 1308 | 1309 | 1310 | 1311 | 1312 | 1314 | 1315 | 1316 | 1320 | 1321 | 1322 | 1323 | 1325 | 1327 | 1328 | 1329 | 1330 | 1331 |
| Days in laboratory before first injection | 5 | 5 | 5 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Number of injections | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 5 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Dose (cc.) | 0.1 | 0.1 | 0.1 | 0.05 | 0.05 | 0.05 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Interval between final injection and killing (days) | 1 | 1 | 8 | 1 | 6 | 8 | 0 | 1 | 1 | 6 | 1 | 2 | 6 | 6 | 7 | 7 | 7 | 8 | 8 | 8 | 2 | 5 | 5 | 5 | 5 | 6 | 6 | 8 | 10 | 10 |
| Ovulation | + | + | + | + | | | | | | L | + | + | + | + | + | + | L | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Development | A | A | — | ? | | | | | | | A | A | ? | ? | — | ? | | A | B | ? | + | + | + | ? | + | + | + | — | + | + |
| Total follicles Normal | | | | | | | | 309 | 108 | 280 | 158 | | 633 | 237 | 396 | 123 | 197 | 267 | 45 | 110 | 215 | 329 | 194 | 83 | 210 | 192 | 76 | 65 | 151 | 95 |
| Total follicles Retrogressing | | | | | | | | 14 | 3 | 4 | 10 | | 3 | 26 | 34 | 4 | 16 | 11 | 3 | 4 | 5 | 18 | 10 | 12 | 20 | 13 | 6 | 5 | 10 | 4 |

EXPLANATION: A, activation but no cleavage; B, developmental block during early cleavage; L, luteinized vesicular follicle; —, egg not developing; +, development apparently normal; ?, no observation.

examined 2, 3 and 5 days after three injections each, and none had ovulated.

*M. lucifugus lucifugus.* It should be noted that ovulation in M. lucifugus lucifugus may follow elevation of temperature during the hibernating period when the animals are brought to the laboratory from the caves (table 2). The earliest record is of three females collected February 6, 1932, and kept 11 days in the laboratory. From then until the middle of March scattered individuals ovulate after 1 to 2 weeks at the higher temperature. After the middle of March an increasing number of individuals ovulate within a few days after collection.

Three individuals, collected December 4, 1935, were injected after 12 days with 0.1 cc. of hypophyseal extract on December 16th, 17th and 18th (table 1, nos. 3, 1283 and 1284). A fourth bat was given two additional injections (no. 1289). An examination of these bats 1 to 6 days after the last injection revealed that none had ovulated and that luteinization of the vesicular follicle had occurred in the animal receiving a total of 0.5 cc. of the extract.

Ten animals, collected January 7, 1936, were injected with 0.1 cc. of hypophyseal extract on January 10th, 11th and 12th. Nine of them ovulated, and in the tenth the vesicular follicle was found to be luteinized (table 1, nos. 1307, 24, 1308-1312 and 1314-1316). Tubal eggs were found in bats nos. 1307 and 24, killed 1 and 2 days, respectively, after the last injection. These eggs contained sperm, had two polar bodies, but cleavage had not occurred. A uterine egg in no. 1310 was undivided after 7 days; only one polar body was found. In no. 1314 a uterine egg had two polar bodies, but the two pronuclei had not united after 8 days. No. 1315 had a uterine egg which had undergone only one division after 8 days. In four specimens killed 6, 7 and 8 days after injections ceased the eggs could not be found. It seems likely that some of them, at least, had disintegrated or been eliminated.

## TABLE 2

*Ovulation in M. lucifugus lucifugus during the hibernating period, conditioned by increase in temperature*

| COLLECTION DATES | DAYS AT 15° TO 20° C. BEFORE KILLING | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | | 7 | | 8 | | 9 | | 10 | | 11 | | 12 | | 13 | | 14 | | 15 | | 16 | | 17 | | 18 | |
| | F. | C. | F. | C. | F. | C. | F. | C. | F. | C. | F. | C. | F. | C. | F. | C. | F. | C. | F. | C. | F. | C. | F. | C. | F. | C. | F. | C. | F. | C. | F. | C. | F. | C. | F. | C. |
| 12/12/31 | 1 | 0 | 4 | 0 | | | | | | | | | | | | | | | | | | | | | | | | | | | 4 | 0 | | | | |
| 12/26/31 | 8 | 0 | | | | | | | | | | | | | | | | | | | | | 2 | 0 | | | | | | | | | | | | |
| 1/14/35 | 9 | 0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2/ 6/32 | 3 | 0 | | | | | | | | | 2 | 0 | | | | | | | | | 3 | 3 | | | 6 | 0 | | | | | | | | | | |
| 2/20/32 | | | | | | | | | | | 2 | 3 | | | | | 6 | 2 | | | 4 | 0 | 5 | 1 | | | | | | | | | | | | |
| 2/20/36 | | | 1 | 0 | | | | | | | | | 1 | 0 | 1 | 0 | | | | | | | | | | | | | | | | | | | | |
| 2/21/35 | 9 | 0 | | | | | | | | | | | | | | | | | | | | | 1 | 0 | | | 0 | 1 | 1 | 1 | | | | | | |
| 2/24/33 | | | | | | | | | | | 0 | 1 | | | | | | | | | | | | | 2 | 0 | | | | | | | | | | |
| 2/28/32 | | | | | | | | | 1 | 0 | 1 | 0 | 1 | 1 | 5 | 4 | 0 | 1 | 4 | 1 | | | | | | | 0 | 2 | 15 | 6 | | | 2 | 5 | 1 | 3 |
| 2/28/37 | | | 7 | 0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3/10/34 | | | 8 | 0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3/14/35 | 1 | 0 | | | | | | | | | 3 | 0 | | | | | | | | | | | | | | | | | | | | | | | | |
| 3/16/31 | 1 | 0 | 2 | 4 | 6 | 2 | 3 | 2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3/23/31 | 3 | 6 | | | 0 | 20 | 0 | 1 | 0 | 7 | | | 2 | 5 | 0 | 9 | 1 | 1 | | | | | | | | | | | | | | | | | | |
| 3/25/32 | | | 1 | 0 | | | | | | | | | 8 | 8 | | | | | | | | | | | | | | | | | | | | | | |
| 4/ 2/32 | 0 | 2 | 1 | 1 | 0 | 2 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

EXPLANATION: F., unruptured vesicular follicle seen in ovary during supra-vital inspection. C., corpus luteum (rupture point) seen during supra-vital examination; in many cases tubal eggs were observed. This material has not been sectioned for microscopic study of development.

On February 20, 1936, ten bats were collected and were injected with 0.1 cc. hypophyseal extract on February 20th, 21st and 22nd. All of these bats ovulated (table 1, nos. 1320-1323, 1325, and 1327-1331), but three uninjected females of the same collection did not (compare table 2). In no. 1320, 2 days after the final injection, there was a four-cell tubal egg. After 5 days all the eggs were uterine, and blastodermic vesicles were found on the tenth day. In no. 1329 the egg was fragmenting, and in no. 1325 it was not observed.

Sections of the ovaries of the bats which did not ovulate were examined. In two M. lucifugus lucifugus (table 1, nos. 1289 and 1312) luteinization of the vesicular follicle had taken place. The oocyte of the luteinized follicle in no. 1289 was surrounded by a heavy membrane and contained atypical cytosomal inclusions. The nucleus contained two large nucleoli. Between the membrane around the oocyte and the zona pellucida, with which the follicle cells were still connected, was a cell which was probably the first polar body. In bat no. 1312, the oocyte within the luteinized follicle appeared to be normal. The cytosomal inclusions were typical, the first polar body had been formed, and the second meiotic spindle was present. In the ovaries of the two M. sodalis (nos. 2 and 1290) in which ovulation did not occur a number of larger follicles in which small antra were present were found, but there were no conspicuously large normal or retrogressing follicles. The same condition was found in three M. lucifugus lucifugus (nos. 3, 1283 and 1284). However, in no. 1284 there was a partially luteinized follicle with traces of an antrum. Retrogression was so extensive that it was impossible to judge how large the folicle might have been when degeneration began. The oocyte in this follicle had formed a polar body.

Cytologically, the ovaries of the injected bats resembled those of normal bats. However, mitotic figures were more frequent in the granulosa cells of follicles in injected bats than in uninjected ones.

### *Growth of follicles*

Counts and measurements of all normal growing follicles and follicles in early stages of retrogression were made on both ovaries of twenty-two injected M. lucifugus lucifugus. The counts for the individuals are given in table 1. Of the twenty-two bats, eleven were young individuals with no wear on their teeth, one had a small amount of wear on one tooth, in nine the teeth were somewhat worn, and one had conspicuous wear on the teeth.

The total number of normal growing follicles in these bats varied from 45 to 633 (average, 203.3). The average number of follicles for animals having no wear on their teeth (eleven bats) was 198.0; for those having some wear (nine bats), 177.4. The number of follicles in early stages of degeneration ranged from 3 to 34 (average, 10.7). For the eleven animals having no wear on their teeth, the average number of retrogressing follicles was 11.5; for the nine bats with some wear on the teeth, it was 11.1.

In figure 1 the individual variation in the percentages of normal follicles of different sizes is shown graphically. The graph indicates that follicles 24 to 43 μ in diameter may constitute from 29.1% to 83.9% of the total normal follicles, and follicles 44 to 83 μ in diameter may vary from 9.7% to 42.7% of the total. Follicles 84 to 123 μ in diameter comprise in the different individuals from 5.2 to 22.8% of the normal group. The variation in follicles 124 to 163 μ in diameter is 0 to 6.1%, and in follicles over 164 μ in diameter it is 0 to 3.2%.

The injected bats were divided into three groups on the basis of the dates on which they were collected. These are compared, in table 3, with control animals killed at corresponding dates. The table shows that in bats collected in December and January and injected, the average number of normal follicles is conspicuously higher than in the controls. In bats collected in February and injected, the average number of normal follicles is somewhat lower than in the controls. There is no marked change in the average number of degenerating follicles in the treated animals.

If the follicles of different sizes are considered, the table shows that the percentage of follicles 24 to 43 μ in diameter is higher in all cases in the injected animals than it is in the controls; the difference is especially marked in the January and February bats. Follicles 44 to 83 μ in diameter are relatively more numerous in the controls than in the injected bats.

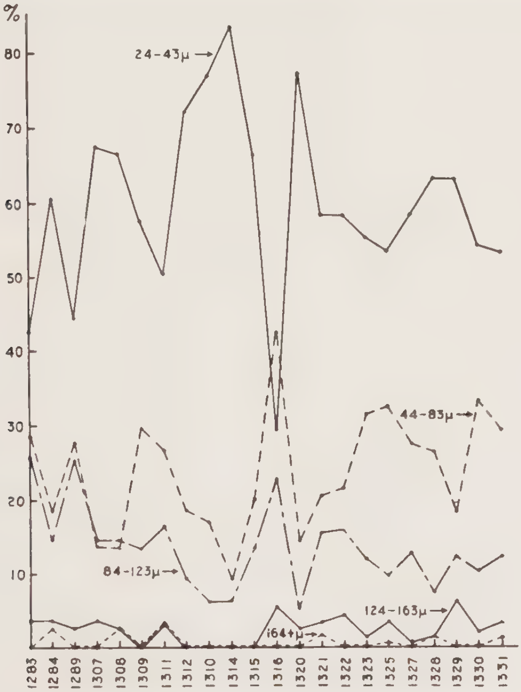

Fig. 1 Graph showing percentage of normal follicles in each size group in injected specimens.

There is no constant increase or decrease in the percentage of follicles 84 to 123 μ in diameter in the injected animals as compared with the normal ones. A very slight increase in the percentage of follicles 124 to 163 μ in diameter is found in the injected bats; if the average number of follicles is calculated for each of the three groups of bats, however, it is found that in each case there are approximately twice as many

## TABLE 3

*Comparison of normal and injected bats (M. lucifugus lucifugus) with reference to numbers and sizes of growing and retrogressing follicles*

| NUMBER AND DESCRIPTION OF BATS, WITH COLLECTION DATES | | DIAMETER OF FOLLICLES (MICRONS) | | | | | | | | | TOTAL GROWING FOLLICLES | AVERAGE GROWING FOLLICLES | TOTAL RETROGRESSING FOLLICLES | AVERAGE RETROGRESSING FOLLICLES |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 24–43 | 44–83 | | 84–123 | | 124–163 | | 164 + | | | | | |
| | | Growing | Growing | Retrogressing | Growing | Retrogressing | Growing | Retrogressing | Growing | Retrogressing | | | | |
| 8 controls | Number | 495 | 444 | | 189 | 19 | 38 | 61 | | 5 | 1166 | 145.7 | 85 | 10.6 |
| (December 21) | Per cent | 42.4 | 38.1 | | 16.2 | | 3.3 | | | | 100.0 | | | |
| 3 injected | Number | 321 | 185 | | 164 | 1 | 24 | 7 | 3[1] | 13 | 697 | 232.3 | 21 | 7 |
| (December 19) | Per cent | 46.1 | 26.5 | | 23.5 | | 3.4 | | 0.4 | | 99.9 | | | |
| 6 controls | Number | 545 | 333 | | 203 | 19 | 11 | 47 | | 4 | 1092 | 182.0 | 70 | 11.7 |
| (January 19) | Per cent | 49.9 | 30.5 | | 18.6 | | 1.0 | | | | 100.0 | | | |
| 9 injected | Number | 1461 | 398 | | 257 | 59 | 32 | 46 | 18[1] | 6 | 2166 | 240.7 | 111 | 12.3 |
| (January 18) | Per cent | 67.4 | 18.4 | | 11.9 | | 1.5 | | 0.8 | | 100.0 | | | |
| 10 controls | Number | 780 | 672 | 2 | 311 | 143 | 20 | 25 | | 1 | 1783 | 178.3 | 171 | 17.1 |
| (March 6) | Per cent | 43.7 | 37.7 | | 17.4 | | 1.1 | | | | 99.9 | | | |
| 10 injected | Number | 965 | 399 | | 192 | 21 | 45 | 68 | 9[1] | 14 | 1610 | 161 | 103 | 10.3 |
| (February 28) | Per cent | 59.9 | 24.8 | | 11.9 | | 2.8 | | 0.6 | | 100.0 | | | |

[1] Follicles with antra.

follicles of this size range in the injected as in the control bats. In the uninjected bats no normal follicles and only a few retrogressing follicles 164 μ or more in diameter are found. In contrast to this, in each group of the treated animals normal follicles with antra and an increased number of degenerating follicles of this size range are present. In figures 2 and 3, the data given in table 3 for the normal follicles in injected and uninjected bats are shown graphically.

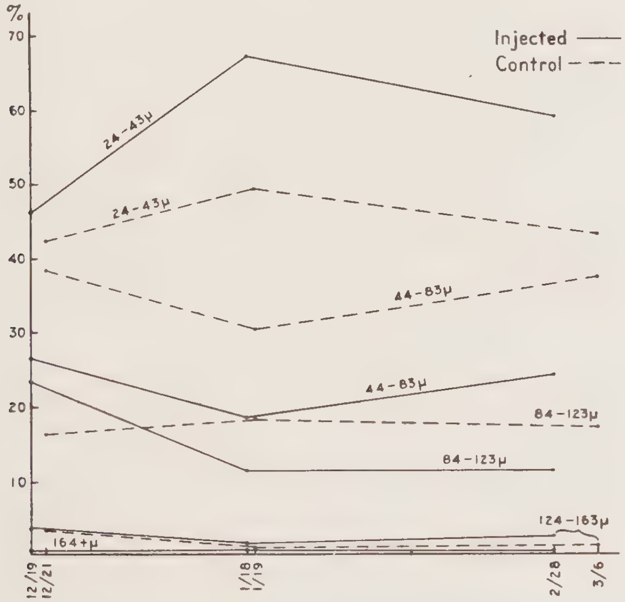

Fig. 2 Graph showing comparison between percentage of normal follicles in each size group in control and injected specimens.

The increase in the relative numbers of follicles 24 to 43 μ in diameter after injection is striking.

The average sizes of the follicles and their contained oocytes were determined in the different size ranges in nine bats injected in January and in ten bats injected in February. Six normal bats killed in January and ten killed in February were used as controls. When the diameter of the oocyte is plotted against the diameter of its follicle, it becomes evident that the oocytes in the follicles of uninjected bats are somewhat larger, except in the group 24 to 43 μ in diameter, than they are in follicles of corresponding size in the treated animals (fig. 4). The ratio of the diameter of the oocyte to that of the entire follicle was determined and compared with the diameter of the follicle. Figure 5 shows that in the control group, the follicle/oocyte ratio decreases slightly until the follicle reaches a diameter of approximately 100 μ. Beyond this size the ratio increases steadily. In bats injected in January and February the ratio follicle/oocyte increases constantly as the follicle becomes larger. The ratio follicle/oocyte is greater for all follicles except those 24 to 43 μ in diameter in the bats given hypophyseal extract than it is in the control animals. Figure 6, in which the thickness of the granulosa has been plotted against the diameter of the oocyte, indicates that in both normal and injected bats the

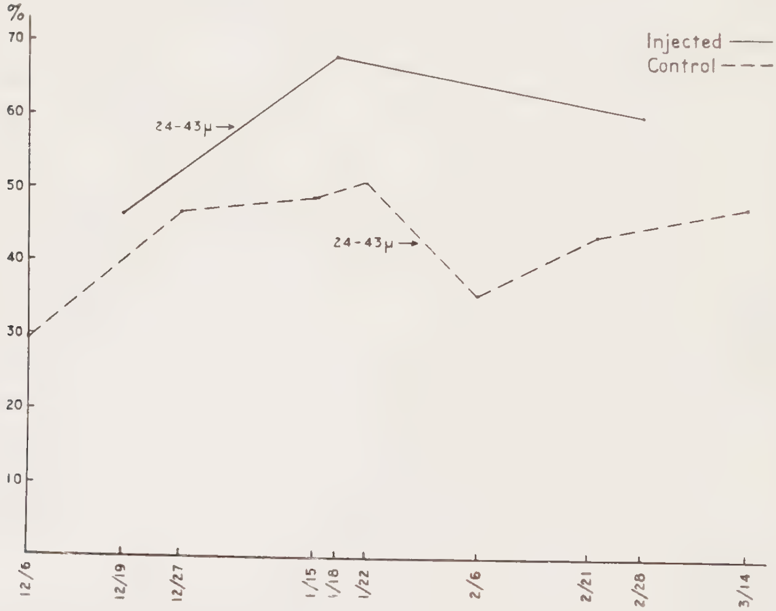

Fig. 3 Graph showing comparison of normal follicles 24 to 43 μ in diameter in control and injected specimens.

follicle cells increase in number as the oocyte grows, but at a more rapid rate in the injected animals.

In the bats which received gonadotropic hormone the cytoplasmic differentiation in the oocytes proceeds in a typical fashion (compare Guthrie and Jeffers, '38 a). Chondriosomes and fat drops are deposited in the cytoplasm, and yolk vesicles appear. All oocytes in follicles with antra are fully differentiated with respect to storage of nutrients in the cytosome. However, limited observations indicate that in the treated animals deposition of yolk vesicles does not occur in the oocyte until it is somewhat larger than in the normal bat.

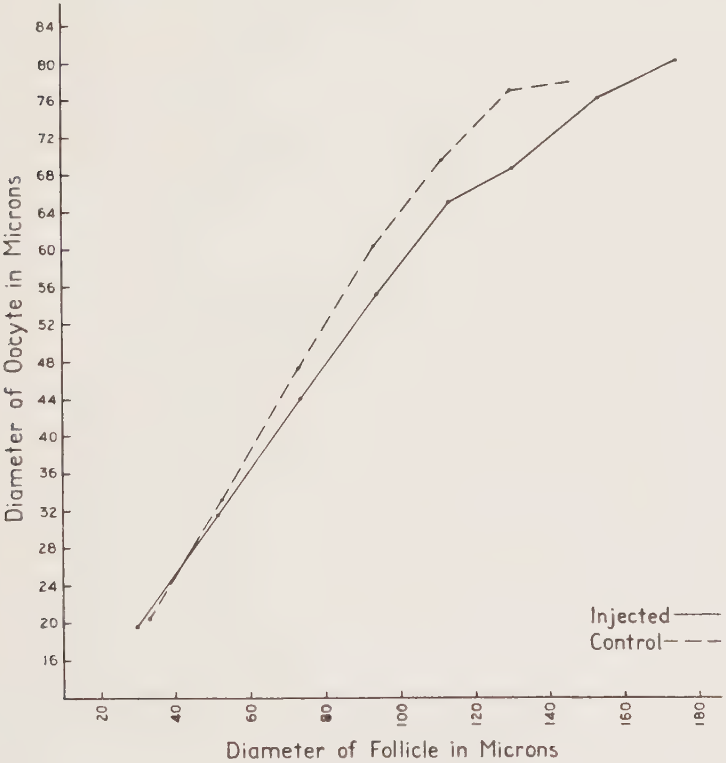

Fig. 4 Graph showing relation of diameters of oocyte and follicle in control and injected specimens.

### *Meiotic spindles*

Pincus ('36) made the suggestion that the first meiotic division of the oocyte might be conditioned by an increase of hypophyseal hormone in the blood. Consequently, oocytes of retrogressing follicles in normal bats and in those injected with hypophyseal extract were examined in order to determine the relative frequency of meiotic spindles. Retrogression of follicles occurs in M. lucifugus lucifugus before the time of antrum formation, but meiotic spindles are frequently found in oocytes in the degenerating follicles (Guthrie and Jeffers, '38 a). Only oocytes which were mature with

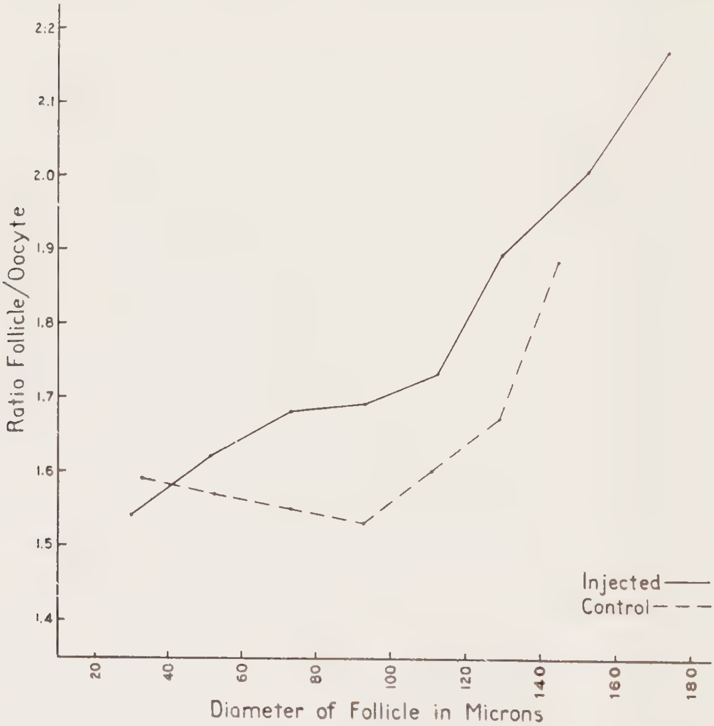

Fig. 5 Graph showing comparison of the ratio of the diameter of the follicle to that of its oocyte with the diameter of the follicle in control and injected specimens.

respect to cytoplasmic differentiation were considered in making the counts.

Both ovaries of ten animals (five collected in January, five in February) injected with three daily doses of 0.1 cc. of hypophyseal extract and killed 5 to 10 days after the last injection were examined. Ovulation had occurred in all except one animal (no. 1312) in which the luteinized follicle contained an oocyte with a meiotic spindle. The follicles were classified into three groups on the basis of the extent of the degeneration of the granulosa, and the oocytes were sorted for the presence of a germinal vesicle or a meiotic spindle. The average number of retrogressing follicles in the group of control bats (five collected in January, five in February) was 19.0, in the injected bats, 14.8. It was found that in the two groups of injected bats the percentage of

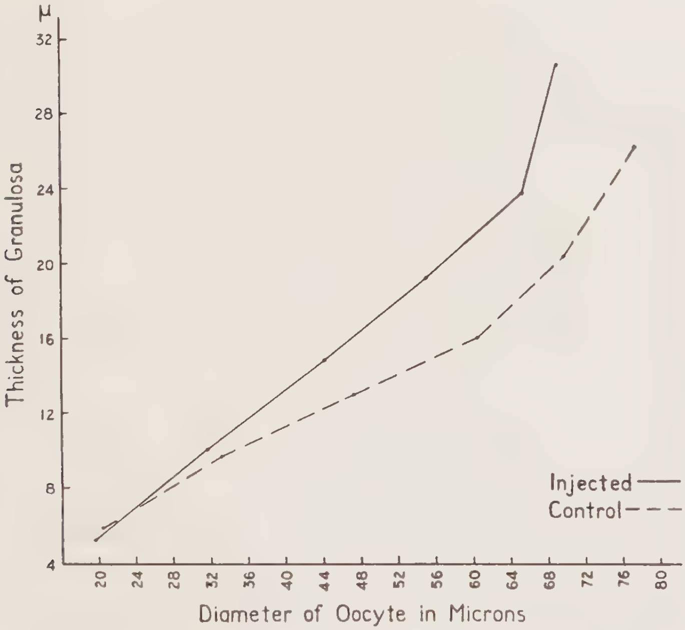

Fig. 6 Graph showing comparison of thickness of granulosa and diameter of oocyte in control and injected specimens.

TABLE 4

*Comparison of normal and injected bats (M. lucifugus lucifugus) with reference to the presence of first meiotic spindles in fully differentiated oocytes of retrogressing follicles*

| NUMBER AND DESCRIPTION OF BATS, WITH COLLECTION DATES | INTERVAL BETWEEN FINAL INJECTION AND KILLING (DAYS) | | FULLY DIFFERENTIATED OOCYTES FROM RETROGRESSING FOLLICLES | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Stage 1 | | Stage 2 | | Stage 3 | | Total | | |
| | | | Germinal vesicle | Spindle | Germinal vesicle | Spindle | Germinal vesicle | Spindle | Germinal vesicle | Spindle | All |
| 5 controls (January) | | Number | 40 | 5 | 2 | 8 | 20 | 5 | 62 | 18 | 80 |
| | | Per cent | 50.0 | 6.3 | 2.5 | 10.0 | 25.0 | 6.3 | 77.5 | 22.5 | 100.0 |
| 5 injected (January) | 7 | Number | 34 | | 12 | 2 | 15 | 7 | 61 | 9 | 70 |
| | | Per cent | 48.6 | | 17.1 | 2.9 | 21.4 | 10.0 | 87.1 | 12.9 | 100.0 |
| 5 controls (February) | | Number | 58 | 3 | 7 | 20 | 11 | 11 | 76 | 34 | 110 |
| | | Per cent | 52.7 | 2.7 | 6.4 | 18.2 | 10.0 | 10.0 | 69.1 | 30.9 | 100.0 |
| 5 injected (February) | 6.2 | Number | 22 | 6 | | | 38 | 12 | 60 | 18 | 78 |
| | | Per cent | 28.2 | 7.7 | | | 48.7 | 15.4 | 76.9 | 23.1 | 100.0 |
| 10 controls (January and February) | | Number | 98 | 8 | 9 | 28 | 31 | 16 | 138 | 52 | 190 |
| | | Per cent | 51.6 | 4.2 | 4.7 | 14.7 | 16.3 | 8.4 | 72.6 | 27.4 | 100.0 |
| 10 injected (January and February) | 6.6 | Number | 56 | 6 | 12 | 2 | 53 | 19 | 121 | 27 | 148 |
| | | Per cent | 37.8 | 4.1 | 8.1 | 1.4 | 35.8 | 12.8 | 81.8 | 18.2 | 100.0 |
| 5 injected (December and February) | 1.4 | Number | 23 | | 17 | 1 | 32 | 5 | 72 | 6 | 78 |
| | | Per cent | 29.5 | | 21.8 | 1.3 | 41.0 | 6.4 | 92.3 | 7.7 | 100.0 |

EXPLANATION: In stage 1 only a few granulosa cells are degenerating; in stage 2 there is conspicuous degeneration of the granulosa; and in stage 3 the granulosa is no longer present, although the relatively typical oocyte and its zona remain.

oocytes containing spindles in these degenerating follicles was lower than it was in the normal bats (table 4). In the ten control bats 27.4% of the oocytes had spindles, while in the injected animals spindles occurred in only 18.2% of the oocytes. It was thought that this difference might be correlated with the fact that almost a week had elapsed between the last injection and the time of killing. Accordingly data on the retrogressing follicles were collected for five animals killed 1 to 2 days after the last injection (table 4). In three of these bats ovulation had taken place. The other two (nos. 1283 and 1284) did not ovulate; their ovaries have been described. In this group of animals, only 7.7% of the oocytes in degenerating follicles contained meiotic spindles.

## DISCUSSION

### *Ovulation and development of ova*

Several European investigators have reported observations on ovulation and development in bats under altered external and internal environmental conditions. Zondek ('33), on December 23rd, found an embryo in a female (species not given) which had been in a warm room (18 to 20°C.) for 14 days after having been kept at 4°C.[4] It was not stated how long these animals had been in captivity. Zondek mentioned that frequently the uterus contained no sperm but that they were observed in the vagina (compare Guthrie, '33 b, pp. 211–214).[5] Zondek also reported that ovulation and fertilization in one bat followed administration of prolan, large doses of which usually induced luteinization of several follicles in each bat. Neither the earliest date at which ovulation was obtained by this method nor the dose given was stated.

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[4] This is much colder than the usual cave temperatures in Missouri (compare Guthrie, '33 a). Ice has been observed in these caves on only one occasion (Rocheport Cave, February 20, 1936) which was after a 16-day period during which the maximum temperature reached 32°F. on only 2 days and a sub-zero (Fahrenheit) minimum was recorded on 10 days; the mean departure from temperatures normal for this period was —20.5°F. (U. S. Weather Bureau records for Columbia, Mo.).

[5] Baker and Bird ('36, p. 149) have misinterpreted these observations. The females were isolated from males and the sperm found in the vagina had been eliminated from the uterus.

Caffier and Kolbow ('34) injected Pipistrellus (? sp.) with prolan in November and found that large follicles were luteinized and none ruptured. Similar results were obtained in Myotis (? sp.) which were injected in December. However, a single Myotis collected December 20th and kept in a warm room without treatment for 3 weeks was found to contain a uterine embryo with notochord and neural tube. Caffier ('34) believed that he induced ovulation in Myotis (? sp.) by injecting large doses of prolan for a period of 2 weeks beginning January 22nd, 2 days after collection; the animals were kept under hibernating conditions. Two days after the final injection an undivided egg was found in the upper part of the tube, and a fresh rupture point was present on the ovary of one individual. A morula was recovered from the uterus of another bat killed 14 days after the last injection. The possibility of spontaneous rupture is not to be overlooked since such a long period of treatment and large dose (a total of 1225 R E of prolan) seem to have been necessary to produce the results obtained. Herlant ('34) injected bats with daily doses of 0.7 to 1.0 cc. of extract of sheep hypophysis (prepared according to Evans' method) or with aqueous extract of fresh human placenta. A specimen of Synotus barbastellus collected December 12th and given five injections contained a two-cell stage in its tract. A single Vesperugo serotinus collected on December 12th was injected for 4 days and killed December 16th; an unactivated tubal egg with second meiotic spindle was recovered. Seven out of eleven individuals (V. serotinus) collected January 20th ovulated after treatment, and development had progressed to early stages of implantation in one individual killed after eight injections.

Our experiments on M. sodalis and M. lucifugus lucifugus show that in these species ovulation can be induced by injection of gonadotropic hormone as early as December 12th and any time thereafter if the animals have not been in captivity for too long a period. Ovulation occurred in one M. sodalis out of three kept in the laboratory 12 days before injections were begun, but none of the M. lucifugus lucifugus ovulated under similar conditions. An examination of their ovaries revealed that there were no normal follicles approaching the size of those which rupture in the spring (compare Guthrie and Jeffers, '38 a). In one animal a luteinized follicle was found. Probably the single vesicular follicle in the other individuals had undergone retrogression.

The minimum amount of gonadotropic hormone necessary to induce ovulation in the bat was not determined. Rupture of follicles occurred in M. sodalis after the injection of 0.5 rat unit of extract and in M. lucifugus lucifugus after a total of 1.0 rat unit had been injected. These doses are apparently much smaller than those used by other workers on the bat.

In the individuals which ovulated after injection of hypophyseal extract during December and January some of the eggs were activated, but development did not take place; internal or early developmental block (Tennent, '22) had occurred. Since spermatozoa had been taken in by some of the ova, the latter were apparently in a fertilizable condition and capable of development. In the bats which ovulated in February, activation of ova and development proceeded normally in the majority of cases. We believe the difference in results can be explained on the assumption that stale sperm [6] present in the tract during the winter are incapable of stimulating normal development and that during February vernal inseminations have begun. It is possible that where activation did not occur sperm had been eliminated from the uteri of the December and January bats, which were kept for several days in a warm room before injections were begun.

[6] The suggested staleness of sperm can be referred to the dilutions in which they occur in the female tract (compare Lillie, '19). Very rarely have sperm been found in the uterus in concentrations in any way comparable to those in the male tract. Usually a relatively dilute suspension of sperm and leucocytes is recovered from the uterus in supravital examinations (compare Guthrie, '33 b).

### *Growth of follicles*

A study of the reactions in the ovaries of immature animals injected with hypophyseal extract and of hypophysectomized animals has convinced a number of investigators that the growth of follicles up to the time of antrum formation is not conditioned by the gonadotropic hormone of the hypophysis (Corey, '28; Smith, '30; Swezy and Evans, '31; Aron, '32; Hill and Parkes, '33; Swezy, '33; Swezy and Pencharz, '33; Selye, '33; Hertz and Hisaw, '34; Selye, Collip and Thomson, '35; Casida, '35; Lane and Greep, '35; Foster, Foster and Hisaw, '37; and Dempsey, '37). Pincus ('36) has stated that the entire growth of the oocyte as well as the early growth of the follicle are independent of hypophyseal hormone.

In contrast to the reports that the ovaries of young animals do not respond to gonadotropic hormone before the time of appearance of follicles with antra, Lane ('35) found that injection of follicle stimulating hormone into young rats was followed by an increase in the total number of follicles. Lane's counts showed that follicles with two or more layers of granulosa cells became more numerous. Cole ('36), who made counts in ovaries of immature rats injected with gonadotropic hormone from mare serum, found no increase in total number of follicles. However, there was a decrease in the number of primary folicles and an increase in the number of larger follicles. Freed and Coppock ('36) have called attention to the fact that, in the guinea pig, all follicles, including the smallest, increase in sensitivity to gonadotropic hormone as the animal matures. Osborn ('38) has reported that the ovaries of adult dwarf mice are much less sensitive than normal ones to gonadotropic hormone. Desaive ('35) found by counting that the number of follicles with two to three layers of cells in the granulosa was almost doubled in the ovary of the adult rabbit after injection of prolan. A consideration of these reports suggests that generalizations about the reactions of follicles to gonadotropic hormone should be based on studies of normal adults of various species.

When adult animals are hypophysectomized the most differentiated follicles begin to undergo retrogression very quickly (24 hours). The larger secondary follicles continue to grow for about 30 days in some cases. Swezy ('33), who made counts the basis of her report, found that there was a conspicuous increase in the number of 'ova' and 'primordial' follicles after hypophysectomy in the rat. Smith ('30) found that growth and early retrogression of small follicles apparently continued for some months after hypophysectomy in the rat. Hill and Parkes ('33) concluded that the atropic condition of the ovary of the anoestrous ferret was only slightly accentuated by the removal of the hypophysis. However, Foster, Foster and Hisaw ('37) found that few primordial follicles remained in the ovaries of the hypophysectomized rabbit after 90 days. Robson ('37) reported that minimal doses of gonadotropic hormone were necessary to maintain the normal reactivity of the ovary of the hypophysectomized rabbit. If the growth of follicles up to a certain stage of differentiation is independent of hypophyseal hormone it is not clear why the ovaries of a hypophysectomized animal should within a relatively short time be without growing follicles. Such an interpretation is based on the tacit assumption that the hypophyseal hormone has a direct effect on the ovary. If the possibility of an intermediary in the relationship or a storage of hormone is granted (compare Casida and Hellbaum, '34), then a varying threshold of response in follicles at different levels of differentiation will rationalize the findings (compare Guthrie and Jeffers, '38 b).

The observations on ovaries of injected M. lucifugus lucifugus indicate that in adult bats follicles respond to hypophyseal hormone before the stage of antrum formation. In the first place, the conspicuous increase in the relative number of secondary follicles 24 to 43 μ in diameter is evidence that growth of the primary follicles has been stimulated. These follicles are much smaller than those in which increases were noted by Desaive, Lane and Cole. Secondly, the larger secondary follicles are stimulated to grow beyond the size which they normally attain before retrogression begins, and small antra appear in them.

A third point brought out by these studies is that the hormone stimulates the growth of the granulosa of small follicles, and indirectly affects the growth of the oocyte. It has been shown that in normal bats killed during January and February the follicle/oocyte ratio tends to decrease until the follicle has reached a diameter of about 100 μ, although there is a gradual increase in the thickness of the granulosa. Materials entering the follicle are apparently used primarily for the growth of the oocyte and for the synthesis of the cytosomal differentiation products. By the time the follicle is somewhat more than 100 μ in diameter the oocyte is approaching its maximum diameter, and subsequently the nutrients entering the follicle are utilized to a greater extent for the growth of the follicle cells. In the injected bats, on the other hand, the follicle/oocyte ratio increases almost uniformly, indicating that the growth of the follicle is occurring at a more rapid rate than that of the oocyte. Additional evidence for the rapid growth of the follicle is derived from the observation that mitoses in very small growing follicles have been seen only in the ovaries of injected bats. Not only is growth of the oocyte somewhat retarded, but cytosomal differentiation progresses more slowly in the oocytes of treated animals. Nutrients coming into the follicle are apparently utilized first for the growth of the follicle, and less material is available for the growth and differentiation of the oocyte.

The follicle/oocyte ratio is lower for follicles 24 to 43 μ in diameter in the injected bats than in the controls. This is correlated with the fact that it is much lower in the injected bats killed during January. In the February bats it is higher in the treated animals than in the controls. It seems probable that these small follicles in the January treated bats are no longer under the influence of the injected gonadotropic hormone, since eight of the nine bats injected in January and used for counts were killed at least 6 days after the last injection; of the February bats, only five were killed 6 or more days after the last injection. Although the number of bats is small, calculations suggest that the follicle/oocyte ratio for follicles 24 to 43 μ in diameter diminishes as the interval between the last injection and the time of killing lengthens.

The data on M. lucifugus lucifugus indicate that in the intact adult bat growth of the follicle at all stages of differentiation and the growth of the oocyte itself are to be correlated with the presence of hypophyseal hormone.

### *Meiotic spindles in oocytes from retrogressing follicles*

It has been shown that the growth and cytoplasmic differentiation of the oocyte are indirectly conditioned by the gonadotropic hormone through its effect on the growth of the follicle. Does the hormone influence in any way the final stages in the nuclear differentiation of the oocyte, the meiotic divisions? Pincus ('36) believed that the production of the first polar body was dependent upon stimulation by hypophyseal hormone, although he did not regard the hormone as acting directly on the oocyte. Moricard ('36) thought that the direct passage of hypophyseal hormone into the oocyte determined the appearance of the first meiotic spindle. Moricard and DeFonbrune ('37) believed that they had induced the formation of the first polar body in vitro by means of hypophyseal secretion. Both Pincus and Moricard explained the formation of meiotic spindles in oocytes from retrogressing follicles on the same basis as in oocytes from normal follicles. However, their conclusions were based exclusively on oocytes from vesicular follicles. In the bat, where retrogression occurs in follicles before the stage of antrum formation, meiotic spindles are frequently seen in the oocytes in these relatively small degenerating follicles. In normal animals, 27.4% of the mature oocytes from degenerating follicles were found to have meiotic spindles, while the percentage of spindles in oocytes from animals treated with hypophyseal extract and killed 5 to 10 days later was conspicuously lower, 18.2%. In bats killed 1 to 2 days after treatment, only 7.7% of the oocytes in degenerating follicles contain spindles. Obviously in the non-vesicular follicle the oocyte, mature insofar as its storage products are concerned, is not stimulated to undergo the first meiotic division but this reaction is repressed by the injection of the gonadotropic hormone. However, since a certain percentage of the oocytes form spindles, complete inhibition does not result from the administration of the hormone. There is as yet no evidence to indicate whether or not the decrease in the injected animals in the number of oocytes that form spindles is to be correlated with the slower rate of cytoplasmic growth and differentiation of these oocytes. The observations of Smith and Engle ('27), who reported that oocytes with typical meiotic spindles were found in retrogressing follicles in hypophysectomized rats, support the hypothesis that hypophyseal hormone does not condition the formation of the first meiotic spindle in oocytes.

The effect of the gonadotropic hormone on follicular degeneration has been noted by several workers. Smith and Engle ('27) reported that after hypophyseal implants into mature animals the number of degenerating follicles appeared to be reduced. Pincus ('36) referred to the atresia-inhibiting effect of the secretion of the hypophysis. Hartman and Squier ('31), however, reported that in the monkey there was a conspicuous increase in the number of degenerating follicles after the implantation of pig hypophyses. All of these investigators made their observations on vesicular follicles. In the bat, the data do not justify the conclusion that there is a significant reduction in the number of retrogressing follicles as a result of injection with hypophyseal extract, but there is certainly no increase during the period studied.

## SUMMARY

1. The bat can be induced to ovulate as early as December by injection of gonadotropic hormone. Spontaneous ovulation correlated with a rise in temperature does not occur before February.

2. Normal development of ova occurs in bats stimulated to ovulate during February, but no reliable instance of typical

development has been obtained after ovulation induced in December and January. This is taken to mean that during the hibernation period the sperm from autumnal inseminations probably lose their capacity to initiate normal development.

3. There is a conspicuous increase in the relative number of the smallest growing follicles, 24 to 43 μ in diameter, after the injection of hypophyseal extract.

4. Growth of secondary follicles beyond the size at which degeneration normally begins and the formation of antra are observed after treatment with the gonadotropic hormone.

5. Growth and cytoplasmic differentiation of the oocyte occur more slowly, in relation to growth of the follicle, in treated than in control bats.

6. Injection of the gonadotropic hormone tends to inhibit the formation of meiotic spindles in mature oocytes from retrogressing follicles.

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# EFFECTS OF THEELIN INJECTIONS IN NORMAL PRE-PUBESCENT CHIMPANZEES[1]

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ONE TEXT FIGURE AND ONE PLATE (EIGHT FIGURES)

Several investigators have shown experimentally that the follicular hormone 'estrin' is capable of producing marked increase in size of the external genitalia of certain primates (Allen, '27; Parkes and Zuckerman, '31; Schoeller, Dohrn and Hohlweg, '32; Zuckerman, '35; Gillman, '37).

The experiments of Zuckerman and Fulton ('34) constitute the only work that has been done on the effects of estrin in the anthropoid apes. They administered, over a period of 20 days, a total of 21,100 r.u. of estrin in oil to an ovariectomized chimpanzee about 8 years old which had previously shown signs of sexual maturity. Slight sexual skin swelling had been observed at irregular intervals for more than a year. The fact that the absolute increase in size of the genital area was quite small—considerably less than one should have expected on the basis of similar experiments with other primates (Zuckerman and others)—probably was due to the very poor physical condition of the subject during the course of injections. Histological examination of uterus and vagina, however, showed the typical estrin injection effects.

The chief aim in most of the experimental work on the relation of estrin and genital swelling in primates has been the determination of effects produced by known quantities of the hormone. For this purpose the subjects used almost invariably have been ovariectomized some time prior to injection. The present experiment was conducted with normal animals, since we did not wish to sacrifice the possibility of using them later as breeding females. Furthermore, there appeared to be some advantage, as a preliminary step, in obtaining information on the effect of estrin in chimpanzees with ovaries intact.

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[1] The writer wishes to acknowledge the assistance of the Committe for Research in Problems of Sex, National Research Council. The advice and cooperation of Dr. Edgar Allen is greatly appreciated. Thanks are also due to Parke, Davis and Company and Dr. Oliver Kamm for supplying the theelin.

The two subjects used in this study were selected in order to have as much difference in age as possible. At the beginning of the experiment, ages and weights of the two were: Lita (no. 70), 6 years, 11½ months, 28.5 kg.; Rosy (no. 68), 2 years, 8½ months, 11.54 kg. Life histories of both subjects are complete. The older of the two subjects, Lita, had exhibited no signs of cyclical genital swelling at the time the experiment was carried out, and even at the present date (March 31, 1938) has shown no appreciable change in this respect.

Injections of theelin in oil (1000 I.U. per cubic centimeter) were made subcutaneously each morning with the subjects' cooperation. Daily records were kept of weight, body temperature, variations in size of the ano-genital area, and modifications of behavior.

## RESULTS

Figure 1 shows the absolute amount of theelin administered to both subjects each day throughout three series of injections. The total amount was the same for both except in the first series, from days 12 to 20, when Rosy received twice the amount given to Lita. This increase is indicated by the dotted line. Rosy received her last injection on the thirtieth day instead of the thirty-third. All photographs were made 24 hours after the last injection, except in the case of Lita in the first series (48 hours before last injection).

The first series of injections was made merely to obtain an approximate determination of the amount of theelin required to produce a minimum reaction. Slight effects were produced even though the daily dose was increased only twice during the 33 days. In the second series approximately the same total quantity was given in one-third the time by increasing the dose daily. In the third series the rate of increase and the total quantity were still greater.

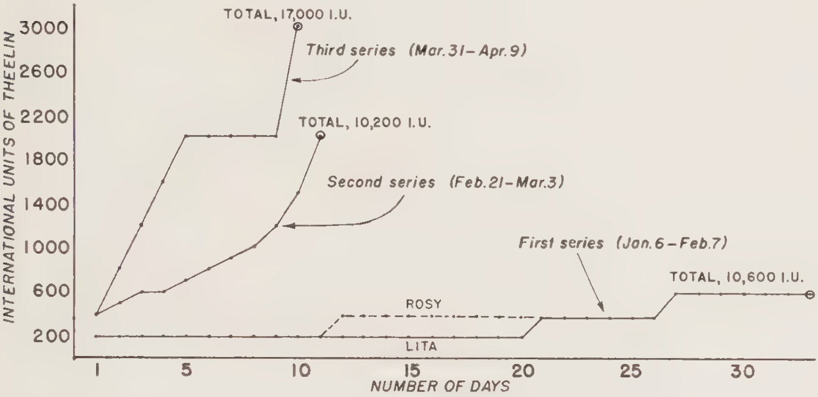

Fig. 1 Amounts of theelin injected daily and the duration of three periods of treatment.

The following abstracts of the protocols describe the effects of the injections. These should be compared with the corresponding figures of plate 1.

Lita (series 1). Two hundred I.U. daily for 20 days produced no appreciable change in the genitalia of this subject, although an increase in general activity was noted by the tenth day. Frequent pulling and manipulation of the nipples also appeared between the fifth and tenth days and was observed frequently until injections were discontinued. Enlargement of the sexual skin did not occur until after the dose had been increased to 600 I.U., 7 days before the injections were stopped.

Rosy (series 1). Two hundred I.U. daily for 11 days had no effect. Dose increased to 400 on the twelfth day. By the nineteenth day there was a slight enlargement of the vulva and a definite increase in size of the nipples. No manipulation of nipples observed. There was marked increase in activity and excitability. Increasing the dose to 600 I.U. on the twenty-seventh day produced a prompt but small increase in size of the pudenda.

Lita and Rosy (series 2). After injection of 1500 I.U. in 3 days both subjects exhibited slight tumescence of the sexual skin. There was a progressive increase in size until the injections were stopped on the eleventh day. Increase in general activity was not as marked as in the first series, although Rosy became unusually friendly and 'presented' frequently to observers.

Lita (series 3). In this series 400 I.U. were given on the first day, and the daily dose thereafter increased by this amount for 5 days. The dose was then held constant until the ninth day, when it was increased to 3000 units. Injections were stopped at this point. The effect was to produce a rapidly increasing swelling which attained a larger maximum than for the previous series. Lita became much more active than before.

Rosy (series 3). At the end of the series, genital swelling in this subject was likewise greater than after series 2 and, except for considerably smaller size, more nearly approached the appearance of the maximum swelling of the mature animal.

First indications of detumescence appeared not later than 48 hours nor earlier than 24 hours after the last injection of a series. It was noted that complete return of the sexual skin to the resting condition required more time than in the normal mature female. Whereas the typical detumescent phase is quite short (Elder and Yerkes, '36), in these two experimental subjects 7 days elapsed between the time of last injection and complete subsidence of the sexual skin.

The results of these preliminary tests show that the normal immature female chimpanzee is quite sensitive to injections of relatively small amounts of ovarian hormone. Contrary to expectation the amount of theelin used, even during the third series, was insufficient to cause menstrual bleeding in either subject. Allen and his associates ('36) demonstrated that experimental menstruation can be induced in immature female rhesus monkeys by injection of approximately 300 r.u. of theelin over a period of 10 days. The menstrual threshold for adolescent animals was slightly lower. If dosage is calculated on a body weight basis, the young chimpanzee, Rosy, during the last series of injections, received from two to three times the threshold dose for monkeys.

Some indication of the importance of the ovarian hormone as a determining condition in estrual behavior is to be found in observations on the stimulus value of the two subjects for a mature male, made 3 days before the end of the third series of injections, when genital swelling was at maximum. A third female, aged 3 years, 4½ months, with the normal infantile condition of the pudenda, was led near the male's cage. He exhibited only casual interest in her. Shortly thereafter Rosy was presented to him in the same manner. An erection appeared promptly upon sight of her, and, as she came near the cage, the male became quite excited, thrust his penis through the wire and finally masturbated. When the older subject, Lita, was presented he continued to be interested but clearly less so than with Rosy. Erection persisted but there was no masturbation. These observations were repeated on the last day of injection with essentially the same results. Such results suggest the desirability of further studies on the role of theelin as a determiner of sexual behavior.

## SUMMARY

Two normal immature female chimpanzees, approximately 3 and 7 years old, were given from 10,200 to 17,000 I.U. of theelin by subcutaneous injection for periods of 33, 11 and 10 days. The most conspicuous effect was the increase in size of the ano-genital region, although there was some response of the mammary glands and definite increases in general activity. Behavioral changes (manipulation of nipples, increase in activity, excitability) appeared before physical changes were manifest. The amounts of theelin employed did not produce menstruation.

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## PLATE 1

### EXPLANATION OF FIGURES

2 and 3 Normal appearance of ano-genital region in subjects Lita and Rosy, January 5, 1937. Camera at 44 inches.

4 and 5 After injection of 10,600 I.U. of theelin in small doses over a period of 30 days. Series 1, February 5, 1937. Camera at 44 inches.

6 and 7 After injection of 10,500 I.U. theelin over a period of 11 days. Series 2, March 4, 1937. Camera at 44 inches.

8 and 9 After injection of 17,000 I.U. over a period of 10 days. Series 3, April 10, 1937. Camera at 40 inches.

Even numbers refer to Lita, odd numbers to Rosy. All photographs by S. D. S. Spragg.

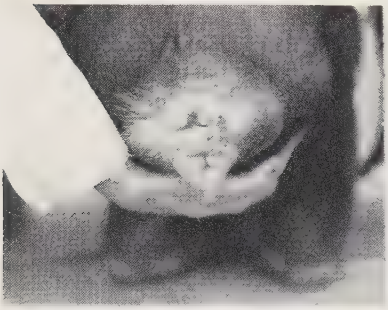

Fig. 2

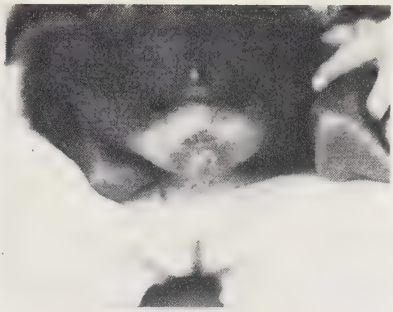

Fig. 3

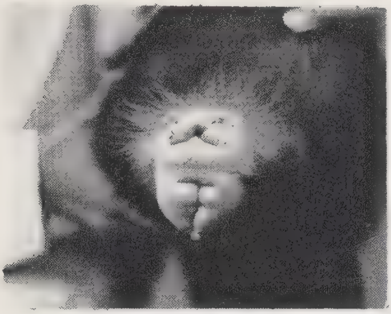

Fig. 4

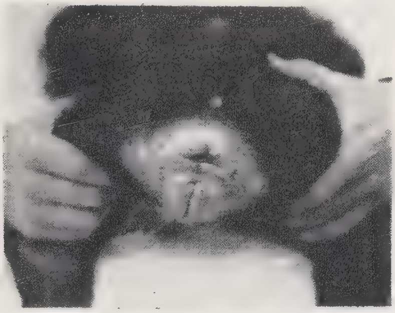

Fig. 5

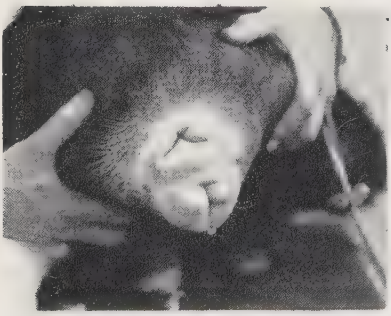

Fig. 6

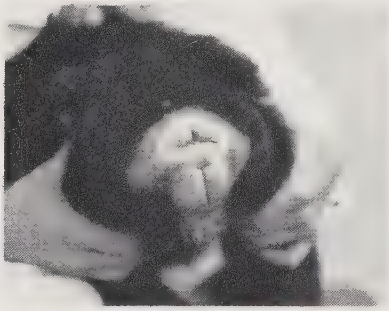

Fig. 7

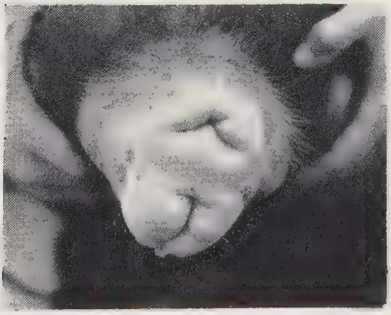

Fig. 8

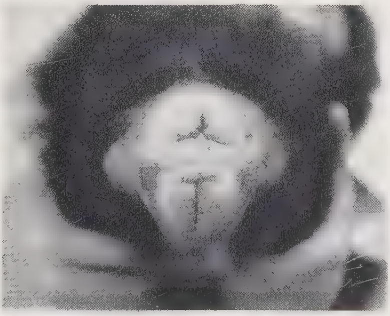

Fig. 9

# THE DIMENSIONS OF THE PELVIC INLET OF 789 WHITE FEMALES[1]

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A very early description of the human female pelvis which included the dimensions of the pelvic inlet was published by William Smellie (1762) in the eighteenth century. He wrote:

> The extent of the brim from the back to the fore-part, commonly amounts to four inches and one quarter; and from one side to the other, the distance is five inches and a quarter: So that this difference of an inch in the different axes, ought to be carefully attended to in the practice of Midwifery.

Later in the same century, Baudelocque (1789), in discussing the dimension of the superior strait, wrote as follows:

> The smallest (diameter), the length of which is in general four inches, extends from the middle of the promontory of the sacrum to the superior and inner part of the symphysis pubis. The largest extends from one side of the strait to the other; and it is ordinarily one inch larger than the preceeding (diameter).

The dimensions of the pelvic inlet as given in numerous obstetrical works of the nineteenth century are in essential agreement with those of Smellie and Baudelocque. Bard's (1812) Compendium of the theory and practice of midwifery, the first textbook of obstetrics published in the United States, contains the following description of the pelvic inlet:

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[1] Read at the meeting of the American Association of Anatomists, Pittsburgh, Pa., March 18, 1938.

[2] This investigation was aided by grants from the Fluid Research Fund of Yale University School of Medicine and from the General Education Board of the Rockefeller Foundation.

Its shape is nearly oval, longest from side to side, and narrower from pubes to sacrum. Its dimensions are in a well-formed pelvis, from side to side from five to five and a quarter inches; and from pubes to sacrum from four to four and a quarter inches on the skeleton, when divested of the flesh and membranes which in the living subject cover the bones, and lessen these dimensions about one inch, but with a lining which will yield considerably to pressure.

In table 1 are listed the dimensions of the pelvic inlet according to six other authors of the same period.

The concept of the shape of the inlet of the female pelvis has not changed materially in the last 200 years. In 1905, a committee of the American Gynecological Society (King, Williams and Davis, '05) published the dimensions of what they termed a 'standard' pelvis. The anteroposterior diameter of its inlet was 11.0 cm. and its transverse diameter 13.5 cm. Twenty years later, Litten ('25) determined the dimensions of the pelvic inlet of 100 German women at autopsy and found an average anteroposterior diameter of 10.4 cm. and an average transverse diameter of 14.0 cm. Jarcho ('33) gives 10.9 cm. and 13.5 cm. as the corresponding average dimensions of the dried pelves of European women, based on five series of German, three series of French, and three series of British cases. According to the same author, an anteroposterior diameter of from 11.0 cm. to 11.5 cm. and a transverse diameter of 13.5 cm. are typical of those pelvic dimensions as given in recent textbooks. Jarcho's figures agree substantially with the descriptions in even the latest editions

TABLE 1

| *Author* | *Year* | *Anteroposterior diameter* | *Transverse diameter* |
|---|---|---|---|
| DeWees | 1828 | "rather more than 4 inches" | "a little exceeds 5 inches" |
| Meigs | 1849 | 10.6 cm. (4.2 inches) | 12.9 cm. (5.0 inches) |
| Luschka | 1864 | 11.0 cm. | 13.5 cm. |
| Hodge | 1866 | 10.1 cm. (4.0 inches) | 13.3 cm. (5.23 inches) |
| Martin | 1866 | 10.7 cm. | 13.6 cm. |
| Verneau | 1875 | 10.6 cm. | 13.5 cm. |

of some widely used textbooks of gross anatomy and of obstetrics, in which the inlet of the normal female pelvis is described as one in which the maximum transverse diameter exceeds the anteroposterior by 2 cm. or more.

It should be emphasized that all the dimensions quoted thus far in this paper were determined on cadavers or on dried pelves. With the development of satisfactory methods of roentgen pelvimetry, it has become possible to measure the pelvic inlet of the living woman with a rather high degree of accuracy. The purpose of this paper is to report these dimensions as determined by Thoms' method of roentgen pelvimetry in approximately 600 primigravid, white women from the obstetrical clinic of the New Haven Hospital and 100 nulliparous, young white women, most of whom were students of the Yale School of Nursing. The student nurses were included in our series in order that we might compare the pelvic dimensions of the clinic patients with those of women from a much more privileged economic group.

The degree of variation in the size and configuration of the superior strait makes it desirable to have some system of classification by means of which pelves can be grouped in accordance with certain easily determinable characters. One of the most practical of these classifications is that devised by Thoms ('37) and Thoms and Wilson ('38) and is based on the relative size of the anteroposterior and the transverse diameters of the inlet. In this system the following types of pelves are recognized:

1. Dolichopellic: The anteroposterior, or conjugate, diameter of the inlet exceeds the maximum transverse diameter.
2. Mesatipellic: The maximum transverse diameter either equals the conjugate diameter or exceeds it by no more than 1 cm.
3. Brachypellic: The transverse diameter exceeds the conjugate diameter by from 1.1 to 2.9 cm.
4. Platypellic: The maximum transverse diameter exceeds the conjugate diameters by 3 cm. or more.

In table 2 the observed percentages of the various types of pelves are listed separately for a recent series of 132 clinic patients, for an earlier series of 450 other women from the same clinic, for 100 student nurses, and for 107 younger girls who range in age from 5 to 15 years. It will be noted that in 37% of the student nurses the anteroposterior, or conjugate, diameter of the inlet was larger than the transverse. The incidences of this type of pelvis among the present and the previous clinic groups were 13.6% and 15.5%, respectively. The transverse diameter of the inlet exceeded the conjugate by more than 1 cm. in only 17% of the student nurses as compared with 40.1% of the clinic women. In only 6% of the student nurses and in only 14.9% of the clinic women was the excess of the transverse over the conjugate diameter more than 2 cm. and thus large enough to fit the textbook description of the normal female pelvis. Obviously, a type of pelvis which is found in only 13.5% of such a large number of cases can scarcely be regarded as typical.

TABLE 2

*Incidence of various pelvic types*

| | *100 nurses* | *132 clinic* | *450 clinic* | *107 children* |
|---|---|---|---|---|
| Dolichopellic | 37.0 | 13.6 | 15.5 | 57.9 |
| Mesatipellic | 46.0 | 43.9 | 45.1 | 33.6 |
| Brachypellic | 17.0 | 34.1 | 34.5 | 8.3 |
| Platypellic | ... | 8.3 | 4.9 | ... |

The observed incidence of the various types of pelves among the student nurses seems especially significant, in view of the superior physical status and the higher economic level of the women comprising that group. More than two-thirds of them were college graduates, and a considerable percentage of the latter were alumnae of Mount Holyoke, Smith, Wellesley, Radcliffe or Vassar. Their superior physical status is indicated by the fact that each of their average bodily dimensions was larger than the corresponding measurement of the clinic women. One got the impression that, as a group, they had attained something at least approximating their maximum normal growth potentialities. In view of these facts the relatively high incidence of dolichopellic and of mesatipellic pelves among them and the rarity of the type usually considered characteristically feminine, suggest that the textbook concept of the latter is in need of some revision.

The data from the younger girls indicate the prevalence of dolichopellic and of mesatipellic pelves during childhood. In table 3 the number of cases and the observed percentage of the various types of pelves are listed separately for each age group. The children studied were residents of one or the other of two New Haven orphanages and closely resembled the clinic women both in economic level and in the nationalities represented by them. It is proposed to repeat the pelvic roentgenograms on these children at about yearly intervals in order to note changes in the size and shape of their pelvic inlet as they approach maturity.

TABLE 3

*The incidence of the various types of pelves in 107 young girls*

| *Age* | *Number of cases* | *Dolichopellic* | *Mesatipellic* | *Brachypellic* |
|---|---|---|---|---|
| 5 | 2 | 100.0% | | |
| 7 | 1 | 100.0% | | |
| 9 | 4 | 75.0% | 25.0% | |
| 10 | 25 | 72.0% | 28.0% | |
| 11 | 21 | 86.0% | 14.0% | |
| 12 | 18 | 50.0% | 33.3% | 16.7% |
| 13 | 14 | 35.5% | 50.0% | 14.5% |
| 14 | 18 | 27.8% | 55.5% | 16.7% |
| 15 | 4 | 25.0% | 75.0% | |

In an attempt to investigate what might be called the functional adequacy of the various types of pelves, we reviewed the case records of 600 white primipara from the obstetrical clinic of the New Haven Hospital and computed the percentage of women of each pelvic type who required some form of operative intervention during delivery. These cases were taken consecutively and without selection, other than to exclude those mothers who gave birth to infants weighing less than 2500 gm. As 'operative intervention,' we included not only caesarean sections, but also version extractions, midplane forceps, and outlet forceps deliveries. In table 4 are listed the number and percentage of women of each pelvic type who were subjected to these procedures, in this series of 600 cases.

The lowest incidence of operative intervention, 16.3%, was in the dolichopellic group, in which the shape of the pelvic inlet differs most widely from that usually regarded as normal. The corresponding values for the mesatipellic, brachypellic, and platypellic groups were 18.0%, 19.5% and 30.7%, respectively. The incidence of caesarean section for the different types was: dolichopellic 0%, mesatipellic 0.7%, brachypellic 4.3%, platypellic 15.4%.

TABLE 4

*Incidence of operative intervention*

| | |
|---|---|
| Dolichopellic type | 15 times or 16.3% |
| Mesatipellic type | 49 times or 18.0% |
| Brachypellic type | 41 times or 19.5% |
| Platypellic type | 8 times or 30.7% |
| Caesarean section | 15 instances |
| Version-extraction | 4 instances |
| Mid forceps | 18 instances |
| Outlet forceps | 76 instances |

If the proof of the adequacy of a given pelvic type is its ability to function efficiently during parturition, the adequacy of the dolichopellic and of the mesatipellic pelves appears to be convincingly demonstrated by our findings. The relatively high incidence of these two pelvic types among the physically superior student nurses is quite consistent with such an interpretation.

## SUMMARY AND CONCLUSIONS

1. The type of pelvis which, for the past two centuries, has been considered normal for white women was found in less than 15% of 582 primiparous, clinic patients, and in only 6% of 100 young women from a much more privileged economic group. It was, therefore, neither the normal pelvis—in the

sense of being the most frequently occurring type—nor was it the most adequate type, as gauged by the relative frequency of operative intervention required during labor by the women possessing it.

2. It has long been known that marked anteroposterior flattening of the adult pelvis may result from severe rickets during early life. The high incidence of round and of anteroposteriorly elongated pelves among the student nurses of our series suggests the possibility that adequate nutrition during early life and other factors which make for the attainment of maximum, normal body size prevent that degree of anteroposterior flattening of the pelvis which has come to be considered as characteristically feminine.

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# ON DARK AND LIGHT CELLS IN THE BRAIN AND IN THE LIVER

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TWO PLATES (TWELVE FIGURES)

Among the characters of neurosecretory cells in the hypothalamus of humans and other mammals (Gaupp and Scharrer, '35) one seems suspect: there are cells staining darker and other staining lighter than the average of the cells of the nucleus supraopticus. In view of the fact that Nissl ('10) demonstrated the artificial shrinkage and inflation of nerve cells and the further fact that the dark cells in the spinal ganglia which Kiss believed to be sympathetic cells turned out to be artefacts (Fisher and Ranson, '34) it seemed advisable to study the causes of the unequal staining of those cells more carefully before ascribing to them a functional meaning. In doing this the literature on a closely related problem, i.e., the question of the dark and light liver cells was encountered. Those cells offered a way to check the results obtained in investigating the supposedly artificial character of the dark and light nerve cells in the supraoptic nucleus.

On the basis of former observations (Scharrer, '33, '34, '37) it was thought that in the liver as well as in the brain, dark and light cells might be brought about artificially by pressing and tearing the fresh tissue when the brain is removed from the skull or pieces are cut out from the liver. It was expected that perfusion of the brain and the liver with the fixing fluid would give different results, as the fixed cell could no longer react in the same way as the fresh material. Brain and liver tissue were therefore compared in this work after fixation by immersion and by perfusion, and the results justified the afore-mentioned suspicion that dark and light cells in both organs are nothing else than artefacts.

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## MATERIAL AND METHODS

Brains of rats, armadillos, opossums and other mammals were studied. Owing to its particular position right around the optic tract the nucleus supraopticus (nucleus tangentialis of Cajal) is liable to be torn, whenever the brain is removed and the optic nerves are cut. Besides that the cells of the supraoptic nucleus are neurosecretory cells and seem to be more sensitive to injurious influences than are other nerve cells. In the first series of experiments brains of opossums were removed in the ordinary way by a transection of the medulla oblongata and subsequent holding of the skull more or less upside down so that the brain by its weight gradually falls out of the skull when the cranial nerves are cut one after the other; the brain finally hangs on the optic nerves and it is then that the changes occur in the supraoptic nucleus during the short time before the optic nerves too are cut. The brain then hangs straight from the skull cavity attached to it only by the olfactory bulbs. Cutting those finally allows the brain to drop into the bottle with the fixing fluid. Brains treated in such a way were compared with others of a second series which were fixed by perfusion. In this case the animal was narcotized with ether or nembutal (pentobarbital sodium), the thorax was opened and the aorta descendens as well as the arteries to the fore limbs were clamped off. A cannula was inserted through the left ventricle into the aorta and was tied in position. Normal saline under 5 feet of pressure was then perfused through the head, in order to wash out the blood. It is important to use not more saline than is absolutely necessary for this purpose (Bodian, '36). For an opossum head 100 cc. are sufficient. Then the fixing fluid is perfused in the same way. It is advisable not to spare the fixative; for an opossum head 300 cc., however, are sufficient. After that the brain is removed and immersed for some hours in the fixing fluid. For the opossum brain Zenker-formol was used. In the same way brains of rats were fixed by immersion and perfusion respectively in different fixatives (Bouin, formol, Zenker-formol). For comparison two brains were fixed in each fluid named, one by perfusion, the other by immersion, as described above. The material was embedded in celloidin and the sections of each pair were stained and dehydrated together in the same dishes to keep all the conditions as nearly identical as possible. There is no danger of confusing the sections of immersed and perfused brains because the blood vessels in the immersed brains are filled with blood whereas the blood is washed out in the perfused.

The same methods were used in preparing livers of rats, rabbits and opossums. In perfusing the liver the thoracic aorta was of course not clamped off. To make sure that no individual differences play a role, in some rabbits and rats one lobe of the liver was tied off in the narcotized animal and pieces were cut out from this lobe for fixation by immersion in formol or Zenker-formol, whereas the rest of the liver was perfused with fixing fluid. Pieces of the liver from the same animal could be compared by this method. All the material was embedded in celloidin (nitrocellulose); the brain sections were 20 μ, the liver sections 10 μ thick. The sections were stained mainly with Heidenhain's hematoxylin; Weigert's iron hematoxylin with van Gieson's stain gives less favorable results. Holmer's ('29) prescription as used and recommended by Ravenna ('32), Böhm ('32) and Clara ('32) was tried too, but with no apparent advantage as compared with Heidenhain's iron hematoxylin.

## RESULTS

*Brain.* In the nucleus supraopticus of rat and opossum brains which were perfused with the fixing fluid all cells look alike (fig. 1). When, however, the brains were removed from the freshly killed animal and were immersed in the fixing fluid, a varying percentage of cells stained darker than the others or appeared even totally black and there were also a few which were clear and light colored as compared with the average (fig. 2). The dark and the light cells in the nucleus supraopticus as well as in other parts of the brain are artefacts due to injuries inflicted upon them during the removal of the fresh brain from the skull.

*Liver.* If we study a section from a perfused rat's liver we find the cells rather uniform in their staining properties (figs. 3, 5). When the material is cut from a liver immediately after perfusion and the fixing fluid not allowed to penetrate for a sufficient time from the blood vessels into the parenchymatous cells, the latter show the effect of mechanical injuries suffered at the cut surface by staining somewhat darker than the rest of the liver cells (fig. 3). But this is inconspicuous compared with the effect of cutting a fresh, unperfused liver of a rat (figs. 4, 6). Here the cut surface is all dark, and beneath it, clusters of extremely dark and light staining cells are found. These cells obviously were injured when the tissue was cut. The dark cells range in all gradations from cells which stain only slightly darker than the rest of the liver cells to extremely shrunken elements which appear deep black. In contrast to these, others display a more or less unstained, swollen cell body and appear much lighter than the average of the liver cells. They too are connected by transitional forms with the normal cell type. The nuclei of the dark and light cells also appear darker and lighter respectively, but are otherwise not different from those of the rest of the liver cells. It is also clear enough from figure 4 how the effect is transmitted through the blood vessels to deeper parts of the organ. Figures 7 to 10 demonstrate that the same conditions obtain in the liver of the rabbit, the experimental animal selected by most of the recent investigators of this problem. It is instructive to compare Ravenna's figures 10 to 12 (pp. 266–267) with our figures 8 to 10, in order to appreciate the identity of the dark and light cells as this author describes them and the artefacts produced in our experiments.

This applies of course in the same way to the work of all other authors quoted in the following pages.

Contrasting pictures of the kind described above can be produced even in the liver of the same animal. When one lobe of the liver of a rat was tied off as described (p. 55) the rest of the liver could be perfused, and pieces of perfused tissue could be compared with those cut out from the unperfused lobe and fixed by immersion. The results are identical with those of the experiments described above in which livers of different individuals were compared. The perfused tissue shows uniform fixation, and no differences in staining properties of the liver cells around the blood vessels are observed (fig. 11); whereas the tissue taken from the unperfused lobe by cutting out pieces from the fresh organ show the effect of mechanical injuries particularly marked around the blood vessels. All those observations clearly point to the conclusion to be discussed in the following section, that the dark and light cells are artefacts.

## DISCUSSION

The foregoing statement is in itself significant only insofar as it indicates once more the value of the perfusion technique in histological methods; that value has, however, already been widely recognized by careful workers. The last paper by Bodian ('37) on the synapse gives impressive examples of the influence of the method of fixation on the final result. Confronted, however, with the vast literature which has accumulated in the last 40 years on the dark and light liver cells, the statement that they are artefacts settles a question on which much work has been wasted. To name a few of the more recent authors there were Rumjanzev ('27), Ravenna ('32), Böhm ('32) and Clara ('31, '32, '33). The latter author also carefully reviewed the literature on the subject and quotes papers by Cohn (1892), Pilliet (1895), Gianelli and Giacomini (1896), Oppel ('00), Browicz ('02), Gilbert and Jomier ('06, '07), Bernard and Loederich ('08), Fiessinger ('11), Berg ('13, '14), Noël ('21, '23), Heinrichsdorff ('24), Holmer ('27, '29), Forsgren ('28), Masachika Kutsuna ('29), Redin ('29), Rabl ('30) and Pfuhl ('32) who all deal more or less extensively with the phenomena of the light and dark liver cells.

The statement that dark and light liver cells are artefacts explains the many contradictory results reported by the workers mentioned above, as well as the constant features in the appearance and localization of the cells on which most of the investigators agree. Thus, for instance, the predilection of the dark cells for the connective tissue and the big veins (fig. 12) is as already pointed out, easily to be understood when one considers the transmission of traction and pressure to the parenchymatous cells through the blood vessels and the connective tissue. It is easy to figure out the effect of cutting through a piece of cake in which some string or the like is baked. Before cutting through the string, the knife pulls it out together with some particles of the cake in its immediate neighborhood which stick to it. In the liver the scissors or the knife pull the blood vessels and the connective tissue before they cut through them and the effect on the liver cells near to them is the same as on the nerve cells of the supraoptic nucleus when the optic nerves are pulled, or on any part of the fresh brain when its surface is pressed. This readily explains the localization of the dark cells around the blood vessels and the connective tissue in the liver of the pig as Rumjanzev ('27) described it and Clara and others have found it in other mammals.

Much work was devoted by different investigators to characterize the dark liver cells. Thus, for instance, their behavior toward vital dyes was studied with the result that Ravenna ('32, p. 262) never found granules of the injected lithium carmin, India ink or pyrrol blue, whereas Böhm ('32, pp. 281–283) found a high grade storage of lithium carmin but none of trypan blue. Clara ('32, p. 227) finally reports a diffuse staining of single groups of dark cells with trypan blue. It is clear that those contradictory observations do not mean a thing as the cells turned to dark ones at random when the liver was cut out for fixation and it is no wonder that the one author found his dark cells full of carmin granules while the other found his cells free of them. It is easily to be understood now why Heinrichsdorff ('24) and Ravenna ('32) considered it as proved that the dark cells produce the bile, whereas Böhm and Clara found that the dark cells have nothing to do with the production of the bile. Terms as 'Tangentialzellen' (Rumjanzev), 'cholopoetische Zellen' (Heinrichsdorff), 'Gallenleberzellen' (Holmer), 'cellule biliari' (Ravenna), etc. which were given to the dark cells can be therefore disposed of. Other controversial statements concerning the contents of glycogen (Rumjanzev, '27; Böhm, '32; Pfuhl, '32, versus Clara), fat (disagreement between Rumjanzev, Redin, Böhm and Clara) and whatnot in the dark and light liver cells nullify each other anyhow. The same is true concerning the relation of the light cells to mitosis (MacMahon, '33, and Clara versus Adler, '04). There is finally no need for a theory concerning the role of the blood supply as a cause of the appearance of dark cells (Böhm, Pfuhl).

As regards the light cells the pathologists have always denied that they have any importance for pathological conditions of the liver (Jaffé, '20; Fischer, '22). There might exist, however, a higher sensitivity of the liver cells in cases of experimental poisoning with phosphorus arsenic, etc. to postmortem changes and to such supravital reactions in cases of mechanical injuries as described above. This would account for the higher rate of dark cells in experiments with poisons affecting the liver as reported by several authors. Likewise, the very irregular occurrence of the cells in question even in the same species (Böhm found among fifty rabbit's livers dark cells only in eleven cases) as well as their highly variable location in the liver, is easily understood if one accepts the point of view suggested above. As artefacts those cells occur at random in any part of the liver, in any animal of the same species and probably in any species. It only depends upon the care with which the piece of the liver is taken out for fixation how many of the dark cells and in which part of the liver they occur. As to more extensive discussions of the cytological details of the dark and light liver cells the reader is referred to the papers of Clara ('31, '32, '33).

Concerning the dark and the light nerve cells little need be said. It has repeatedly been shown by various workers that dark sclerotic nerve cells can be brought about artificially (Nissl, '10; Droogleever Fortuyn, '26–'27; Scharrer, '33, '37; Fisher and Ranson, '34; Cox, '37). It may here be added that light nerve cells are artefacts produced in the same way as the light liver cells. But this statement as such does not solve the problem, it only changes its significance and shifts the aim of future studies. It is no longer a problem what dark and light cells mean in regard to the functional status of the nucleus supraopticus in the brain or of the liver because those cells do not exist in this form in the living organism. No functional meaning can be attributed to them, since they are artefacts. The problem concerns rather the rapid reaction which the cells can show in the interval between removal from the organism and the fixation as a consequence of mechanical and probably also other injurious influences. A complete breakdown of the colloidal system of the cell seems to occur with instantaneous loss or imbibition of water, resulting in dark stained shrunken cells or in those which are swollen and light. Also Clara ('32) assumed such changes in the colloidal status of the cells with the only difference that he regarded them as cells occurring in the liver intra vitam, as did all the former authors also. It seems worth while to study those supravital reactions more extensively as they might reveal something concerning the ways in which living cells may react to environmental influences.

## SUMMARY

Dark and light cells in the brain and in the liver of mammals are artefacts due to pressing of the parenchymatous cells and pulling of blood vessels, nerves and connective tissue. Their appearance can be avoided by fixation by way of intravascular perfusion of the organs with the fixing fluid.

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## PLATE 1

### EXPLANATION OF FIGURES

1 Nucleus supraopticus of a rat's brain after perfusion with Zenker-formol. Celloidin, 20 $\mu$, Heidenhain's iron hematoxylin.

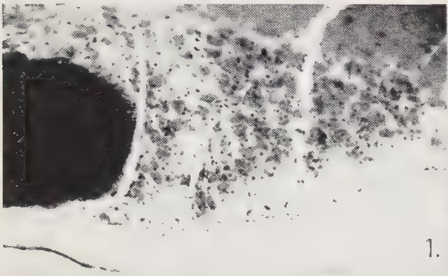
2 The same after fixation by immersion in Zenker-formol.

3 Cut surface of a rat's liver after perfusion with Bouin's fluid. Celloidin, 10 $\mu$, Heidenhain's iron hematoxylin.

4 The same after fixation by immersion in Bouin's fluid.

5 One part out of figure 3 at higher magnification.

6 One part out of figure 4 at higher magnification.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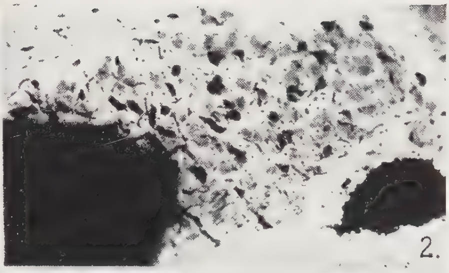
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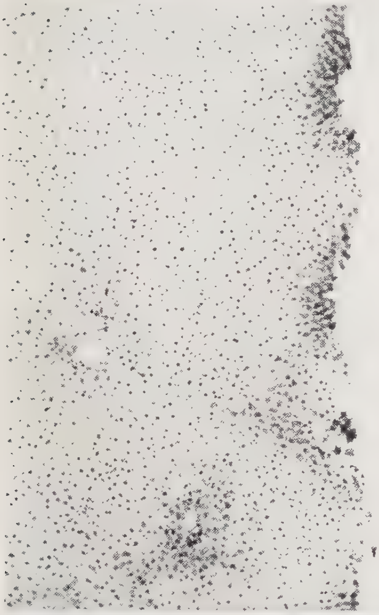
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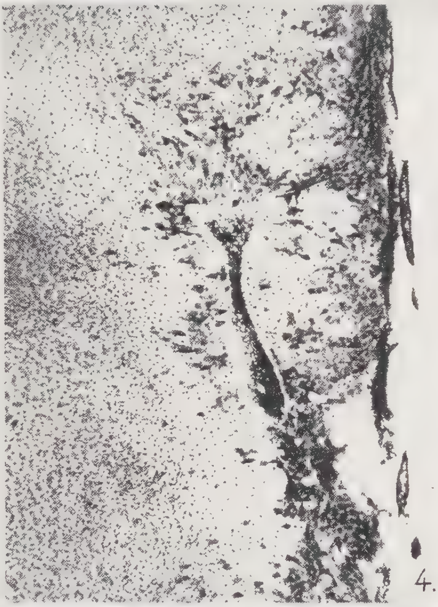
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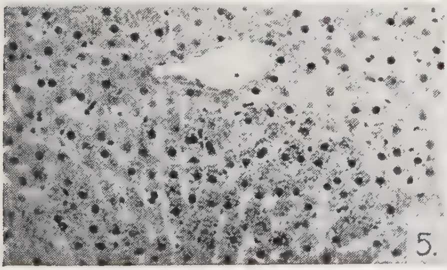
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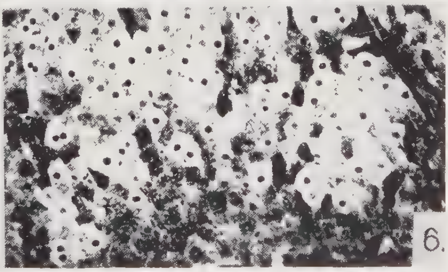
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## PLATE 2

### EXPLANATION OF FIGURES

7 Rabbit's liver near the cut surface after perfusion with 20% formol. Celloidin, 10 $\mu$, Heidenhain's iron hematoxylin.

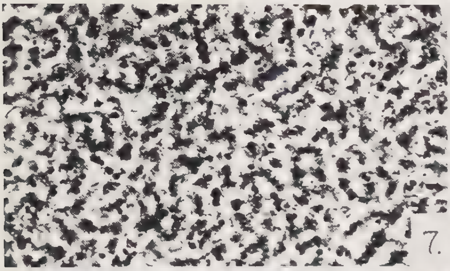
8 The same after fixation by immersion in 20% formol.

9 One part out of figure 7 at higher magnification.

10 One part out of figure 8 at higher magnification.

11 Rat's liver perfused with Zenker-formol. Technique as in figure 7.

12 From a piece of a lobe of the same liver as figure 11 tied off before perfusion and fixed by immersion in Zenker-formol.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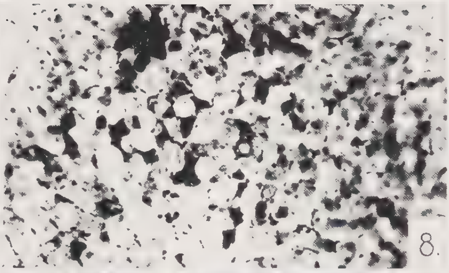
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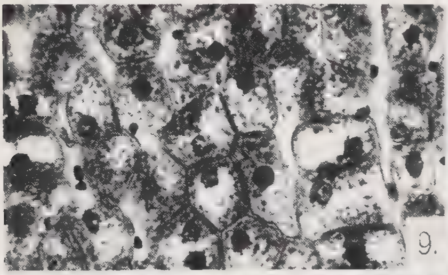
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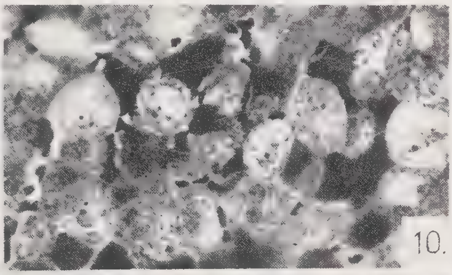
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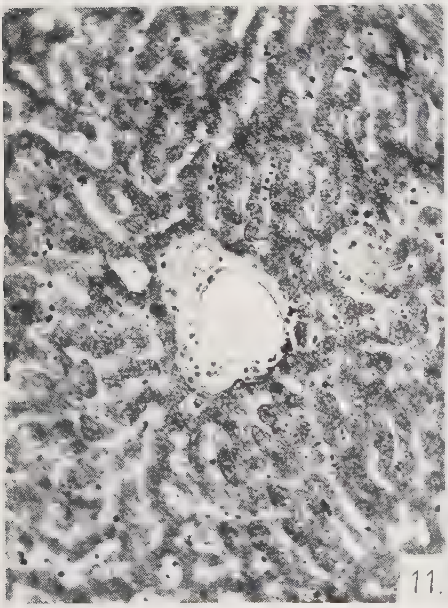
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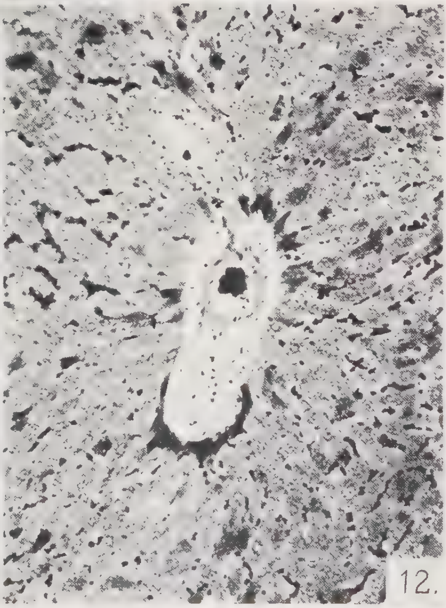
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# THE ORIGIN OF THE PENILE URETHRA AND BULBO-URETHRAL GLANDS WITH PARTICULAR REFERENCE TO THE RED SQUIRREL (TAMIASCIURUS HUDSONICUS)[1]

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TWO PLATES (TEN FIGURES)

Critical examination of the current descriptions of the development of the penile urethra and bulbo-urethral glands, both in textbooks and in the original papers, leaves one with the impression that the following questions are still not answered satisfactorily:

1. What is the origin of the urethral plate; is it ectodermal or endodermal?

2. Does the urethral plate contribute the whole penile and membranous portions of the urethra, or only the part in the glans, or perhaps only the ventral wall or floor of the penile urethra?

3. Does the cavity of the penile urethra form by folding together of the genital folds over the so-called urethral groove, or does this fusion result in a solid plate which later hollows out, or is the groove merely a result of ingrowth of the ectoderm to form the plate which later becomes canalized by dissolution of its inner epithelial cells, a common process in development of such cavities?

4. In view of the latter possibility, are the urethral groove and so-called primitive urogenital ostium the same thing, and does this ostium ever connect with the lumen of the urethra in the male? That this ostium does connect with the urethral

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[1] This work was aided materially by a grant from the Wisconsin Alumni Research Foundation.

lumen in the human embryo sometime between 14 and 17 mm. is one of the statements which is rather uniformly made and for that reason should perhaps not be called into question, yet our material indicates that such a connection never exists in the squirrel.

5. Is the perineal raphe which extends from the urethral orifice to the anus in the adult the result of the closure of the urethral folds; or is it the remnant of the line of ingrowth of the urethral plate from the ectoderm, left as the plate becomes separated from the surface ectoderm, a process which actually does begin just in front of the anus and extends to the tip of the penis?

6. Are the bulbo-urethral glands of endodermal or ectodermal origin?

In addition to these general problems, the question of the manner of development of the male urethra and accessory glands in the red squirrel was of particular interest in view of the studies made by Mossman, Lawlah and Bradley ('32) on the adult male reproductive tract of the squirrels. As can be seen from the following quotation of the summary of their paper, there are certain peculiar relationships of the accessory glands and their ducts; one of them concerns the prostate which sometimes opens in common with the ductus ejaculatorius, another is the single penile duct draining the bulbo-urethral and bulbar glands, and a third is the marked dissimilarity between the genital system of the red squirrel (Tamiasciurus hudsonicus) and that of all the other species. They summarize as follows:

> The typical sciurid reproductive tract is characterized by the presence of a pair of coiled, relatively little-branched seminal vesicles (vesiculae seminales): a simple, compact, highly compound tubular prostate gland (glandulae prostaticae) opening by a single pair of primary ducts; and a pair of very large compound tubular Cowper's glands (glandulae bulbo-urethrales) with wide spiral ducts which constrict as they enter the corpus spongiosum of the bulb, but immediately after entering expand and then unite forming at this region of union a well-developed gland, the bulbar gland

(glandula bulbi), of very different character of epithelium from that of the true Cowper's gland. This bulbar gland drains through a single glandular penile duct which runs distalward through the corpus spongiosum ventral to the urethra to near or beyond the ventral flexure of the penis where it joins the urethra from the ventral side. This peculiar arrangement of the Cowper's ducts is the most characteristic feature of all the genera, except Tamiasciurus where there is no suggestion of it. Here the seminal vesicles and prostate are like those of the others, but the Cowper's glands are minute, slightly compounded, tubular glands entirely enclosed within the sheath of the corpus spongiosum of the bulb and opening by an extremely short non-glandular common duct into the ventral side of the urethra at the distal end of the bulb. There is also in this genus a large, non-glandular, ventral urethral diverticulum or sinus in the bulb (sinus urethrae bulbi), while no urethral sinus exists in the other species. An os penis is present in all except Tamiasciurus. All the genera seem to lack ampullary glands (ampullae) and para-urethral glands.

These findings raised the question whether the accessory glands as found in the common red squirrel are homologous to those of the other Sciuridae, and also, whether those of the typical Sciuridae are homologous to the glands of other mammals.

A. H. Siddiqi ('37) studied the development of the penile urethra, penile duct, bulbar gland, and bulbo-urethral glands of the spermophile (Citellus tridecemlineatus). He came to the conclusion that the penile urethra is formed from the dorsal portion of the urethral plate, this plate having formed as a distal prolongation of the pars phallica of the urogenital sinus and being thus entodermal in origin. On the other hand he believes that the penile urethra distal to the entrance of the penile duct, the penile duct, bulbar gland and the bulbo-urethral glands are derived from the ventral one-half of this urethral plate; this ventral one-half, he thinks is formed from the external cloaca ('ectodäum' of Van der Broek or 'estodermal cloaca' of Keibel). Therefore, according to him, the distal end of the urethra, the penile duct, bulbar gland and the bulbo-urethral glands are of ectodermal origin. His conclusions were based on a study of male spermophile embryos of 11, 13, 17, 19, 27 and 35 mm. crown-rump length.

According to Hall ('36) the penile urethra of the mouse is ectodermal and the urethral sinus entodermal.

Debierre (1883) thought the bulbo-urethral glands arose by evagination or outpouching of the epithelium of the urethra but made no attempt to determine the germ layer origin of this portion of the urethra.

Keibel ('04) studied male Echidna embryos and described an ectodermal portion of the cloaca from the cranial end of which the bulbo-urethral glands develop, beginning as thickenings of this ectodermal epithelium. During this same year a paper was published by Herzog ('04) in which he described the portion of the penile urethra proximal to the ostium urogenitale primitivum as being derived from that part of the phallic portion of the urogenital sinus which contains a lumen and the portion distal to this opening as forming by a splitting of the urethral plate to form a groove. This urethral plate is a distal, solid, epithelial prolongation of the phallic portion of the urogenital sinus. Felix quotes Herzog in Keibel's Human Embryology, vol. II, page 963, as follows: "This trough-like groove begins to close from the basal side, the closure taking place not at the margins of the opening where the ectoderm and entoderm meet, but at the middle of the groove; the wide groove is thus divided into a closed tube, the prolongation of the pars phallica of the shaft, and a shallow groove." Thus according to this work, the entire penile urethra and, consequently, its glandular derivatives are entodermal in origin.

Lichtenberg ('06) studied a number of human embryos and says the penile urethra and the bulbo-urethral and Skene's glands are of ectodermal origin. Eggerth ('15) criticized Lichtenberg's work, claiming that he accepted without verification the report of the ectodermal origin of the bulbo-urethral glands in the Monotremata and Marsupialia and then made these findings directly applicable to man. This 'report,' which Lichtenberg supposedly accepted, apparently was the volume on the male sex organs by Disselhorst ('04) in Oppel's Lehrbuch der vergleichenden miroskopischen Anatomie der Wirbeltiere.

Van der Broek ('08) studied male embryos of the kangaroo, Halmaturus, and described an ectodäum (Keibel's 'ectodermal cloaca') from which the bulbo-urethral glands arise just distal to the point where the entodermal portion of the cloaca joins the ectodermal portion.

Felix' account of the development of this portion of the urogenital system appeared in Keibel and Mall's Human Embryology ('10). He states with a great deal of certainty, that the entire penile urethra is, without any reasonable doubt, of entodermal origin and that therefore all glandular structures derived from this part of the urethra are also entodermal. Felix admits that he did no work on this problem himself but merely stated Herzog's ('04) views. He was so vigorous in his statement that few have been inclined to reopen the argument since.

## MATERIALS AND METHODS

The materials used were obtained from the collection of red squirrel embryos of the late Dr. T. G. Lee, University of Minnesota, and from our own collection of red squirrel and chipmunk embryos. These specimens were fixed in either Zenker's or Bouin's solution. Serial paraffin sections 10 μ thick were prepared (some cut in a transverse plane and others in a sagittal plane) and were stained with either iron hematoxylin, hematoxylin and erythrosin, or hematoxylin, erythrosin and orange G. These sections were studied and tracings of certain sections showing important structures were made; also graphic reconstructions of the bulbar and penile urethra and the bulbo-urethral glands and ducts were made in sagittal and horizontal aspect. Blotting paper models of the entire urethra, part of the wolffian and müllerian ducts, part of the ureters, the rectum and anus and the anterior body wall between the umbilicus and the tail process, were made from two of the embryos. Using an arc lamp microprojector, the sections magnified 120 times, were projected onto blotting paper. Both the free or lumenward border and basement membrane of the epithelia of the structures under investigation were outlined. At this magnification it was found that two thicknesses of blotter were required to represent the thickness of each section. These tracings were then cut out by means of a special cutting tool (Cutawl, International Register Company, Chicago, Illinois). They were then stacked and glued. After the glue had dried, the model was painted with a thin solution of celluloid in acetone to size and give added strength. This was followed with three coats of white enamel.

TABLE 1

| *Embryo from red squirrel female no.* | *Length (C-R)* mm. | *Graphic reconstructions* | *Tracings* | *Models* |
|---|---|---|---|---|
| 51 | 7.0 | 0 | 0 | 0 |
| 1 | 7.5 | 0 | 0 | 0 |
| 48 | 11.5 | 0 | 0 | 0 |
| 6 (Lee) | 16.5 | 0 | 0 | 1 |
| 13 (Lee) | 20.5 | 3 | 7 | 1 |
| 73 | 29.0 | 0 | Several | 0 |
| 2 (Lee) | 31.0 | 0 | Several | 0 |
| *Chipmunk female no.* | | | | |
| 94 (Lee) | 13.5 | 0 | 0 | 0 |

One of the embryos modeled had been sectioned in a transverse plane. The second model was made from an embryo sectioned in a sagittal plane. By studying the contour of the ducts as seen in the latter embryo, it was possible to construct the former model more correctly as there were no straight-line landmarks to guide one in stacking these blotting paper sections.

The nomenclature followed is the same used by Mossman, Lawlah and Bradley ('32).

Table 1 lists the material studied.

## DESCRIPTION

The crown-rump length of the embryos is given in table 1. This is only an approximately accurate criterion of age since some of the embryos were curled more than others and so as a supplement to this a description of the state of development of certain external features seen on gross examination is given along with the description of the urogenital system of each.

The 7.0-mm. embryo is one in which the anterior limb buds show the first flexure whereas the posterior limb buds are still simple bulges. This embryo shows a series of well-developed mesonephric tubules and a complete mesonephric duct which empties into the urogenital sinus. The rectal portion of the cloaca is entirely separated from the urogenital portion. The ureteric buds are diverticula, 110 μ in length, from the wolffian ducts. The genital ridge shows no sex differentiation.

In the 7.5-mm. embryo the first flexure is seen in both the fore and hind limb buds. The anterior limb buds show the constriction between the lower leg and a paddle-like foot. The individual digits are not recognizable as yet. The posterior limb buds show no such paddle-like extremity. The mesonephroi and wolffian ducts are completely developed and do not show any signs of involution. The urogenital sinus is differentiated into a bladder portion, a urethral portion (pars pelvina) and a pars phallica. The phallus shows signs of beginning elongation and the urethral plate is present. The ureteric buds still empty into the wolffian ducts and the very early metanephroi consist of the pelvis with primary and secondary branches. The genital ridge is still in the indifferent stage.

In the 11.5-mm. embryo the axial condensations of mesenchyme which represent the future digits are distinct in the paddle-like extremities of both the fore and hind limbs. The hair follicles of the vibrissae are distinct. The first tubercle of the pinna of the ear is just beginning to show. This embryo was poorly mounted, and several sections were torn or lost. However, this did not interfere seriously with study of the genital system. The mesonephroi show early signs of involution and the wolffian ducts empty into the urethra distal to the neck of the bladder. The ureters join the urethra just below the neck of the bladder. Glomeruli occur in the metanephroi, but there is little or no specialization of the different portions of the uriniferous tubules. The testis can be recognized as such by the sex cords which are just beginning to form the unmistakable early tubules. The urethral plate is thicker in its deepest portion than in that part which extends from this deepest portion to the ventral surface of the phallus. It is interesting to note that the membranous urethra joins the plate at a distinct angle. This indicates that the urethral plate is not a distal extension of the phallic portion of the urogenital sinus. At this region of junction there is as yet no certain indication of the anlagen of the bulbo-urethral glands.

The extremities of the fore limbs of the 16.5-mm. embryo show the digits well separated while those of the hind limbs are just beginning to separate. The pinna of the ear is definite. The eyelids are present but are wide open. Hair follicles are present over the general body surface. This male embryo was reconstructed in blotting paper. The mesonephroi show fairly well marked evidences of transformation into the epididymis. The wolffian ducts enter the urethra 335 μ distal to the neck of the bladder (fig. 10) while the müllerian ducts fuse about 500 μ from the urethra and the fused ducts then end blindly 100 μ from the urethra. The ureters empty at the neck of the bladder. The tip of the phallus is just beginning to bend caudally (figs. 9 and 10). At the distal end of the ventral surface of the phallus a small epithelial tag marks the distal termination of the urethral plate. The membranous urethra joins the urethral plate at an angle of about the same degree as in the 11.5-mm. embryo. The urethral lumen has extended a short distance into the plate (fig. 10). The bulbo-urethral glands are simple, sac-like evaginations from the proximal end of the urethral plate and extend dorsally and slightly laterally from this point. There is no expansion of the most distal portion to indicate a differentiation between the gland body and duct (fig. 10).

The 20.5-mm. embryo is slightly more advanced than the 16.5-mm. stage. The digits of both the fore and hind feet are well separated. The eyelids are still wide open. This embryo was reconstructed both graphically and in blotting paper. The blotting paper models (figs. 6 and 7) show the wolffian ducts entering the dorsal side of the membranous urethra approximately 450 μ distal to the neck of the bladder. The müllerian ducts lying medial to the wolffian ducts fuse and end blindly 60 μ from the urethra. The ureters empty into the lateral walls of the neck of the bladder. At this stage of development the proximal one-half of the urethral plate has separated from the ventral surface ectoderm of the phallus and has formed the proximal portion of the penile urethra. The lumen of this part of the urethra is cylindrical, but the epithelium has a peculiar arrangement. The ventral and lateral epithelial walls of the urethra are only 2 to 3 cells thick and follow the contour of the lumen, but in the middle of the dorsal wall there is a solid epithelial ridge (figs. 2, 3 and 4) which, if traced toward the membranous urethra seems to be directly continuous with it (fig. 1). The large lumen of this portion of the penile urethra ends proximally by evaginating on either side to form the two bulbo-urethral gland anlagen (fig. 1). Consequently the lumen in the membranous portion of the urethra has to dip sharply ventrally to meet and join the lumen of the penile portion. The distal one-half of the urethral plate still lies in contact with the surface ectoderm (fig. 8) and its distal termination is indicated externally by the epithelial tag (fig. 5). This portion contains no lumen. The phallus of this stage is considerably elongated and bent sharply caudally (figs. 5 and 6).

The 29.0-mm. embryo has developed nails on both the fore and hind limbs. The pinnae of the ears have nearly attained their adult form and the eyelids are closed. In this embryo the anlagen of the seminal vesicles consist of L-shaped evaginations from the wolffian ducts; the blind ends of these anlagen are bent cranially. The anlagen are 120 μ long. The bulbo-urethral glands are dorsal evaginations of the proximal end of the penile urethra. The evaginations are differentiated into glandular and duct portions and the two portions together are approximately 350 μ long. The prepucial ingrowth of epithelium is extensive. The urethral plate has separated from the surfaces nearly to the tip of the penis and a very narrow urethral lumen can be traced to within about 70 μ of the deepest part of the urethral groove which is located practically at the position of the definitive external urethral orifice. The epithelial tag is still present.

The 31.0-mm. embryo shows only one marked external feature distinguishing it from the 29.0-mm. stage, this being a much thicker skin which has become opaque and which shows heavy wrinkling. This embryo is almost full term. Here the seminal vesicles have a very peculiar structure in that they are T-shaped evaginations from the wolffian ducts. The top of the 'T' is parallel to the wolffian duct and is connected to it by the stem of the 'T.' Thus, one end of the top of the 'T' projects cranially while the opposite one projects caudally. The over-all length of the anlage is 280 μ. The mesonephros is almost completely transformed into the epididymis in this embryo. The two vasa deferentia almost fuse just as they join the urethra, and their lumina are enlarged at this point. Since no ampullary glands (ampullae) are described for the various genera of the Sciuridae, just what these dilatations of the vasa deferentia represent is questionable. The bulbo-urethral glands themselves are not much larger in this than in the 29-mm. embryo, but the ducts are considerably longer so that the combined lengths of the ducts and of the glands is 550 μ. The urethral plate is canalized to the distal end; thus the definitive external urethral orifice is now present and the epithelial tag and urethral groove have disappeared.

The 13.5-mm. chipmunk (Tamias striatus) embryo shows external characters which are very similar to those seen in the 11.5-mm. red squirrel (Tamiasciurus hudsonicus) embryo. The one noticeable difference is that the digits of the chipmunk embryo are slightly more separated. This chipmunk embryo shows an early stage in the development of the metanephroi which is comparable to the stage seen in the development of the 11.5-mm. red squirrel embryo. The ureters empty into the wolffian ducts just before the latter empty into the urethra. The urethral plate is continuous from its junction with the membranous urethra to the epithelial tag. The membranous urethra joins this urethral plate by making a sharp bend ventrally just as was seen in the 11.5-mm. red squirrel embryo. At the point of this junction the bulbo-urethral glands arise as dorso-lateral evaginations of the proximal end of the urethral plate.

## DISCUSSION AND SUMMARY

The penile urethra of the red squirrel develops from the urethral plate by a method somewhat different from that which was described by Herzog ('04). The urethral plate certainly develops in large part from an ingrowth of ectoderm along the ventral or urethral surface of the phallus. However, at the proximal end of the plate, where it is continuous with the endoderm of the urogenital sinus, the deep edge may possibly be of endodermal origin, and may correspond to the solid distal extension or pars phallica of the urogenital sinus which Herzog described. This proximal deep portion for a time remains a solid epithelial ridge while the urethral lumen is extending from the sinus into the more superficial thickened portion of the plate (figs. 1 and 2).

The formation of the penile urethra from the urethral plate begins with the thickening of that portion of the plate which lies in contact with the surface ectoderm in the region of the primitive urogenital 'ostium' or groove and for some distance in front of it (fig. 4). Since the 'ostium' or urethral groove is at first merely the more ventral portion of the proctodeum which is separated off from the anal portion by the urorectal fold, it follows that this thickening of the urethal plate takes place first at the proximal end and proceeds distally as the phallus and plate form and the groove itself moves distalward. This thickened superficial edge of the plate is at first solid. As the urethral groove moves forward the plate behind it separates from the surface ectoderm by thinning and finally dissolution of the epithelial layer connecting the thickened portion to the surface. Because of this, examination of a series of cross sections from the groove proximally gives the impression that the thick urethral anlage is migrating toward the deep edge of the plate, but this is probably not true. The lumen of the penile urethra begins to form at the junction of the already present lumen of the urogenital sinus (the membranous or muscular urethra) with the urethral plate, and proceeds distally. The central epithelial cells of the thickened urethral anlage undergo dissolution, often leaving temporary strands and septa crossing the lumen (fig. 3). In none of the fetuses studied had lumen formation kept pace with the distad migration of the urethral groove or so-called 'primitive urogenital ostium,' and so no actual 'ostum' existed. The presence of such a connection with the 'ostium' or groove is almost necessary to account for hypospadias, but one cannot help wondering if it ever occurs in normal embryos before the groove has reached the tip of the penis. The sloughing of the epithelial tag which is known to occur would then be concomitant with the arrival of the groove at that point, and with the establishment of the definitive urethral orifice. (See the description of the 31-mm. embryo.) If this is what actually happens, the condition in the female could be largely accounted for by the failure of the urethral groove to move distalward, in other words by an arrest or slowing of the process of ingrowth of ectoderm to form the urethral plate. The position of the distal end of the urethral lumen is indicated on figures 6, 7 and 10.

We are therefore inclined to believe that the penile urethra is developed either entirely from the ectodermal portion of this plate; or, if the deep, proximal, possibly endodermal portion of the plate contributes anything to it, it must be only to the upper (dorsal) one-half or roof of the urethra. The bulbo-urethral glands and their ducts in the red squirrel are formed as dorso-lateral diverticula from the proximal end of the ectodermal portion of the urethral plate. In the chipmunk, no doubt, the bulbar gland and penile duct are also derived from this ectoderm, although in the stages which were studied these structures are not yet present. This corresponds to the belief held by Siddiqi ('36), who has studied the development of the bulbo-urethral glands, their ducts, the bulbar gland and the penile duct in the spermophile (Citellus tridecemlineatus).

The seminal vesicles appear sometime between the 24.0-mm. and 29.0-mm. stages in the red squirrel as posterior diverticula from the vasa deferentia. After proceeding a short distance dorsally, the diverticula bend and continue cephalad parallel to the vasa deferentia. In the 31.0-mm. specimen which was the oldest embryo studied an interesting condition was noted. Instead of each of the diverticula having a single cephalad branch, there is also a caudal branch so that the complete anlage is T-shaped. Gross ('05) thought that probably the seminal vesicle and prostate of the squirrel developed from a single evagination of the vas deferens, the seminal vesicle developing from a cephalad branch of this evagination and the prostate from the caudal branch. Since we have been unable to find any indication of a developing prostate in any of the embryos studied, including the 31.0-mm. embryo which is almost term, it is possible that Gross' assumption was correct. Neither of the chipmunk embryos which were sectioned showed any indication of seminal vesicle or prostatic anlagen. Before one can be sure of the origin of the prostate it will be necessary to study late foetal and newborn specimens up to the point where a definite and unmistakable prostate is seen.

The prostatic utricle is not seen in any of the embryos which were sectioned. However, the fused müllerian ducts are present and while the fused portion varies considerably in length in these embryos, the important fact is that the caudal end of their fused ducts is separated from the prostatic urethra by a layer of mesoderm which ranges from 60 to 100 μ in thickness. There is no indication even in the latest stage studied of the comparatively well-developed prostatic utricle which is seen in the adult red squirrel.

## CONCLUSIONS

On the basis of the evidence presented the following tentative conclusions may be drawn concerning the development of the genital tract of the red squirrel:

1. The urethral plate is mainly derived from an ingrowth of ectoderm beginning just in front of the cloacal opening and extending to the tip of the phallus. The deep proximal portion of the plate may be derived from the entoderm of the urogenital sinus.

2. The penile urethra seems to be derived in its entirety from the urethral plate.

3. The lumen of the penile urethra is formed by a dissolution and separation of the inner cells of the epithelial cord formed from the urethral plate. It is not produced by closure of the genital folds over the urethral groove.

4. In the red squirrel the urethral groove seems to be the same as the urethral groove and primitive urogenital ostium described for man, but at no stage which has been examined is there a connection with the lumen of the urethra. Probably such a connection does not occur in this species until the urethral lumen reaches the tip of the phallus, at which time the groove disappears and the definitive external urethral orifice is formed. This latter condition occurs in the 31.0-mm. embryo which is practically full term.

5. The perineal raphe is the remnant of the line of ingrowth of the surface ectoderm to form the urethral plate and is formed as the plate separates from the surface. This separation first takes place just ventral to the anal pit. It exists only between the urethral groove and the anus. The groove is the external indication of the region where separation of the urethral plate from the surface ectoderm is taking place. The fact that this raphe extends to the urethral orifice in the adult indicates that the urethral groove is moved distally until it finally reaches the tip of the penis and that the major part of the urethra is therefore ectodermal.

6. The bulbo-urethral glands of the red squirrel are so situated during development that it is reasonably certain that they are outgrowths of the ectodermal part of the urethra.

7. An evagination which seems to be the seminal vesicle takes place from the urethral end of each vas deferens between the 24- and 29-mm. stages.

8. No indication of the prostate gland or prostatic utricle is present at full term.

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## PLATE 1

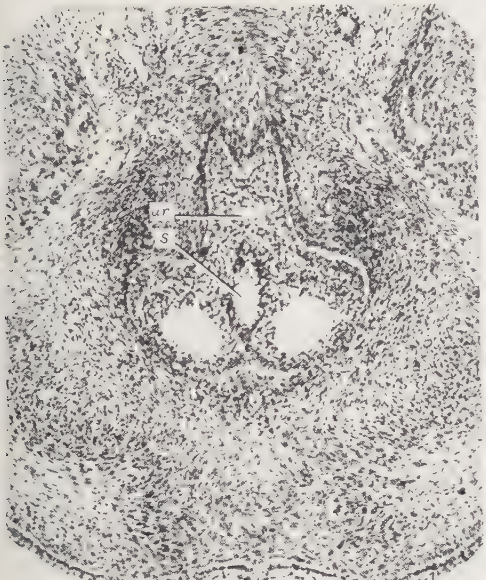
### EXPLANATION OF FIGURES

1 Embryo, 20.5 mm. Section of the urethra (ur.) and bulbo-urethral glands at level indicated by highest mark on figure 5 and lowest on figure 6. The cavities of the two bulbo-urethral glands are seen below and lateral to the membranous urethra. What appears to be a fourth canal lying immediately below the lumen of the membranous urethra is the mesenchymal septum (S.) between the two glands. × 80.

2 Embryo, 20.5 mm. Section of the urethra at level of the second mark from top on figure 5 and second from bottom on figure 6. This shows the communication between the lumina of the membranous and penile portions. The lumina of the two bulbo-urethral glands which are separated by a mesenchymal and epithelial wall in figure 1 are fused in this section and continue distally from this point to form the lumen of the penile urethra. The epithelial ridge above the lumen holds the position which is occupied by the membranous urethra in figure 1. × 80.

3 Embryo, 20.5 mm. Section of the penile urethra at level of the third mark from the top in figure 5 and third from the bottom on figure 6. It shows the dorsal epithelial ridge decreased in size from that seen in figures 1 and 2. The lumen of the urethra is completely divided by a trabecula of epithelium which, in following the complete series of this portion of the urethra, is seen to be a membrane forming a sinus within the urethra; the sinus ends blindly at its distal extremity but there are a number of irregular fenestra in the proximal portion of the membrane which allow communication between the lumen of the rest of the urethra and this sinus. This membrane is probably a transitory remnant resulting from the dissolution of the central epithelial mass of the early urethra. In the mid-line of the perineum the epithelium shows a ridge externally and a groove on its deep surface. Both the ridge and the groove extend from the anal pit to the point where the urethral plate is still in contact with the surface ectoderm (figs. 5, 6, 7 and 8) and indicate the former line of attachment of the urethral plate. × 80.

4 Embryo, 20.5 mm. Section of the phallus at the level of the lowest mark in figure 5 and highest in figure 6. No lumen is present in either level of the urethral plate. Both portions of this plate are intimately attached to the surface ectoderm. The more proximal section through this plate (the upper one) shows the superficial edge beginning to widen, to form the penile urethra from the urethral plate. × 80.



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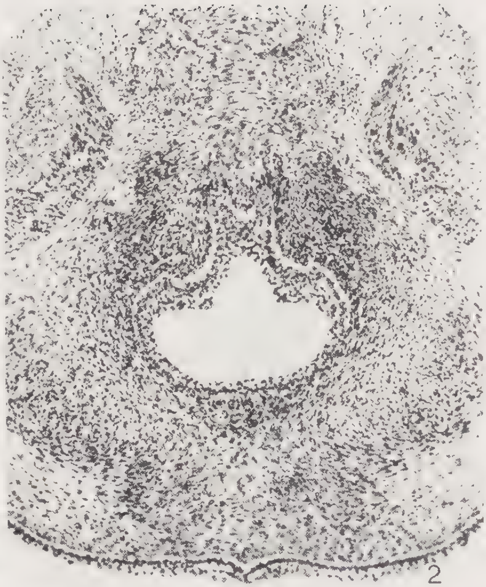

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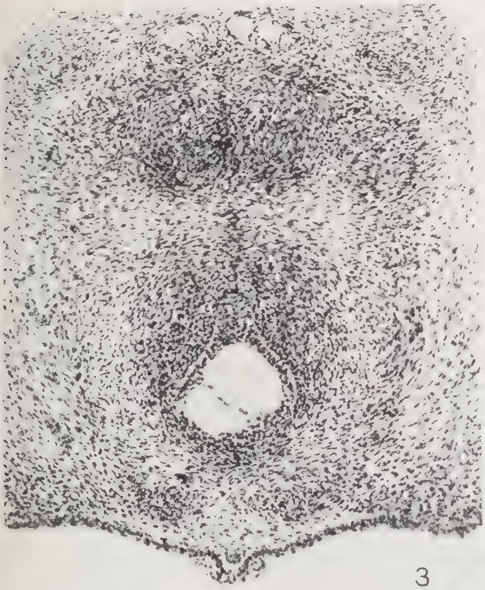

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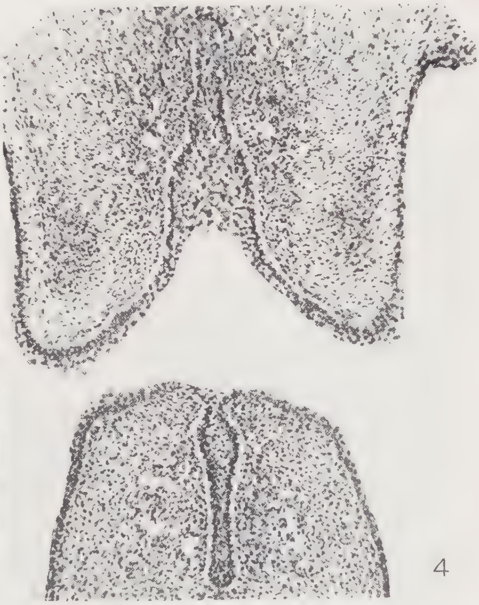

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## PLATE 2

### EXPLANATION OF FIGURES

5 Model of 20.5-mm. embryo. Perineal surface of the model showing the phallus at the lower left. The 'primitive urogenital ostium' (o.u-g.p.) lies in the midline between the phallus and the body wall. The anal pit (a.p.) lies near the top of the model in the midline. Between the primitive urogenital ostium and the anal pit is a slightly elevated ridge in the surface ectoderm which is the external indication of the former attachment of the urethral plate. The four black marks, counting from above downward, indicate the levels of the sections shown in figures 1, 2, 3 and 4.

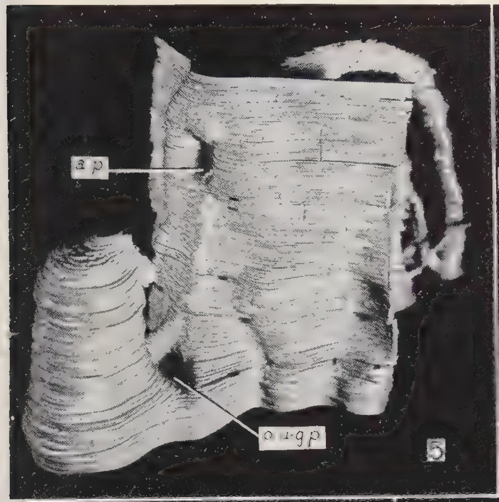
6 Model of 20.5-mm. embryo. The rectum (re.) is shown curving from the left downward and toward the right to end in the surface ectoderm. Just to the right of the inner cut end of the rectum is the neck of the bladder with the urethra (ur.) extending distally from it. The two ureters (u.) empty into the neck of the bladder and the wolffian (d.w.) and müllerian (d.m.) ducts approach the urethra some distance below the bladder. At the junction of the membranous and penile portions of the urethra the bulbo-urethral glands (g.b-u.) extend laterally and backward. A groove (ra.) running superiorly from the anus in the midline indicates the former line of attachment of the urethral plate and the future perineal raphe. The distal end of the urethral lumen is indicated by the asterisk. The four marks, counting from below upward, indicate the levels of the section shown in figures 1, 2, 3 and 4.

7 Model of 20.5-mm. embryo. The model is inverted so that the uppermost surfaces of the model actually represent the most caudal portion of the embryo. the position of the wolffian (d.w.) and müllerian (d.m.) ducts and the ureters (u.) in relation to each other and to the urethra are best shown in this view of the model. The end of the urethral lumen is indicated by the asterisk.

8 Model of 20.5-mm. embryo. This view was taken from above looking caudalward. The undifferentiated urethral plate (u.pl.) is seen extending distally into the phallus. This view also shows how the bulbo-urethral glands (g.b-u.) extend lateral and posterior from the penile urethra.

9 Model of 16.5-mm. embryo. Perineal surface. The left one-half of the phallus is shown with a portion of the anterior body wall between the umbilicus and the tail process. The epithelial tag on the caudal surface of the phallus indicates externally the internal distal termination of the urethral plate. The 'primitive urogenital ostium' (o.u-g.p.) and anal pit (a.p.) are also shown.

10 Model of 16.5-mm. embryo. Deep surface. The bladder (v.u.) is seen in the lower part of the photograph with the allantoic duct to the left and the neck of the bladder to the right. The ureter (u.) is seen at the neck of the bladder, and the wolffian ducts enter the urethra some distance distal to the neck of the bladder. The fused müllerian ducts approach the urethra between the wolffian ducts. The junction of the membranous urethra and urethral plate (u.pl.) lies between the two bulbo-urethral glands (g.b-u.). The rectum (re.) curved so much to the left that only a small part was within the region modeled. The distal end of the urethral lumen is indicated by the asterisk.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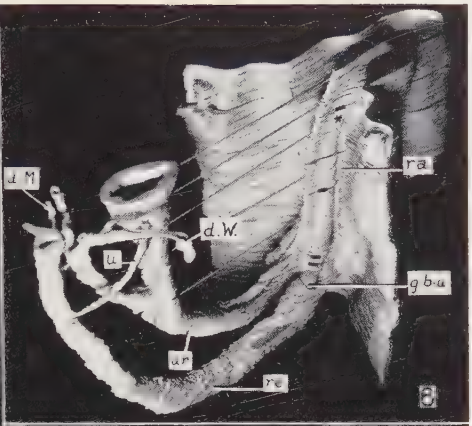
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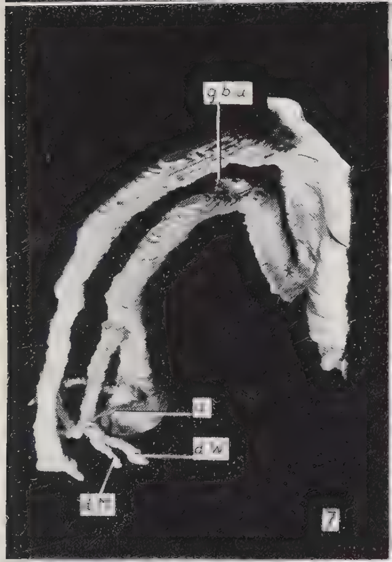
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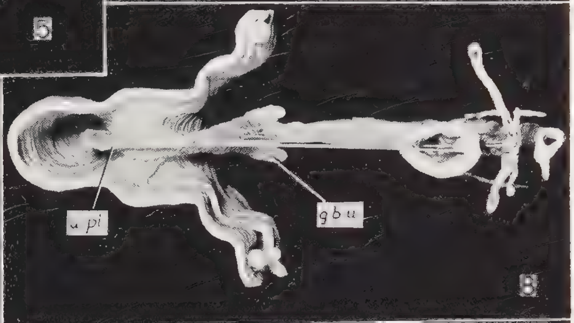
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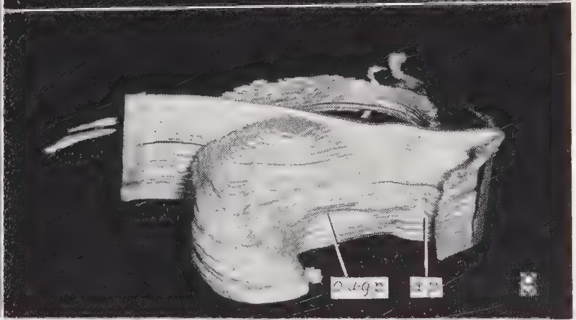
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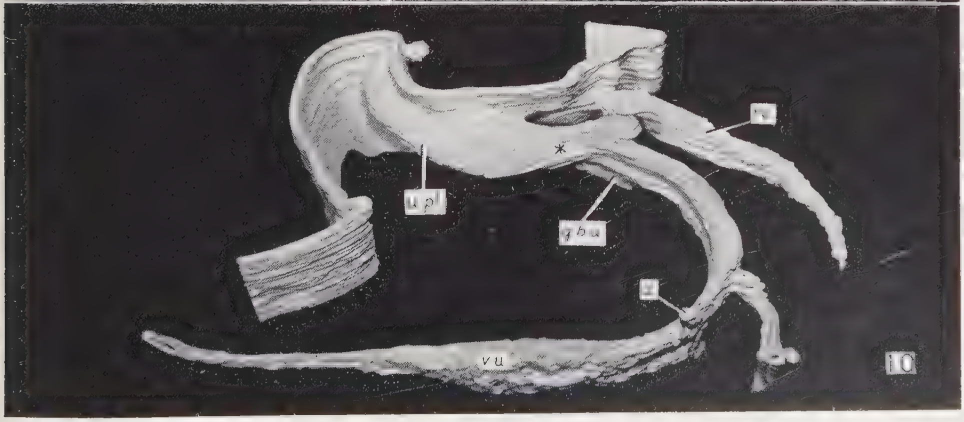
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# STUDIES ON THE REPRODUCTIVE SYSTEM OF THE ALLIGATOR

## III. THE ACTION OF TESTOSTERONE ON THE ACCESSORY SEX STRUCTURES OF RECENTLY HATCHED FEMALE ALLIGATORS

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ONE PLATE (FOUR FIGURES)

### INTRODUCTION

The action of sex hormones when administered to reptiles has received relatively little attention. The effects of folliculin injections in the turtle were reported by Kehl in 1930. Since that time communications have appeared on the results of administration of various female sex hormones to embryonic and to adult lizards (Turner, '35; Clapp, '37; Dantchakoff, '37 a). Regamey ('35) has studied the effects both of homoplastic testis transplants and of injections of macerated testis into castrate male and female lizards of the genus Lacerta. Risley ('37) has published a preliminary report on the results of injections of testosterone propionate into the eggs of a turtle, Chrysemys bellii.

Following the author's study of the effects of oestrone administration to immature alligators (Forbes, '38), it seemed desirable to investigate the action of testosterone in this reptile, since it has been frequently reported that the male hormones are capable of effecting striking changes in the reproductive systems of vertebrates. Attention will be chiefly centered in this paper on the action of testosterone on the female accessory sex structures. The problem of possible transformations induced by male hormone in the accessory organs of the male and in the gonads of both sexes requires further investigation; the results will be reported at a later time.

## MATERIAL AND METHODS

The twenty-four alligators used for this experiment were approximately 3 months old when injections began. Control and experimental animals were maintained in separate cages and fed plentifully with raw beef muscle and liver.

The experimental animals were injected intraperitoneally with a 3.5% alcoholic solution of testosterone, a portion of the stock solution being diluted as needed for this purpose. Injections were made on five occasions during the first 2 weeks of the experiment and were made daily, 6 days a week, for the next 11 weeks. During the 91-day period each animal received a total of 10 mg. of testosterone divided into seventy-two equal doses. One injected animal died during the experiment.

On the day following the last injection all control and experimental animals were killed. The reproductive tracts were preserved in Bouin's fluid. Serial sections were cut at 10 μ and stained with Mayer's acid hemalum and eosin.

The testosterone used in this experiment was supplied through the courtesy of Dr. Erwin Schwenk of the Schering Corporation.

## RESULTS

### *A. Macroscopic observations*

At autopsy it was found that the control group was composed of three males and six females, while the experimental group consisted of one male and thirteen females. The unequal sex ratio was due to the fact that it is difficult, if not impossible, to distinguish sex externally in alligators of this age. Comparison of control and experimental females revealed in the latter group a marked and consistent hypertrophy of the oviducts, which had increased both in diameter and in length (fig. 2). As a result of the increase in length the ostial half of each duct, partially restrained by the mesosalpinx, was thrown into a flattened spiral or 'herring bone' position. Hypertrophy of the cloaca at that level at which it was joined by the oviducts was noted.

There was no increase in size of the clitorides of the injected females as compared to those of the control females. It may be noted that the penis of the single experimental male, in marked contrast to the penes of the control males and to the clitorides of all the females (as observed both in this and in other experiments), was so greatly enlarged as to protrude from the cloacal opening (fig. 1).

### *B. Histological observations*

The oviducts in the control females were conspicuous and well formed. These structures, lying lateral to the mesonephros and joined to the base of the latter by a mesosalpinx, possessed a smooth mucosa of simple columnar epithelium (fig. 3). A connective tissue stratum in which muscular elements could not be recognized comprised the greater part of the oviduct wall. Oviduct and mesosalpinx were both invested with peritoneum which continued onto the free lateral surface of the mesonephros.

In both male and female control animals the mesonephros appeared as a flattened body lying in contact with and immediately dorsal to the gonad and consisting for the most part of a wolffian duct and a system of tubules. Occasional glomeruli were still present.

Histological examination revealed that the lumina of the oviducts of the experimental females were conspicuously larger than in the controls (fig. 4). The mucosal cells of the experimental oviducts were taller and so crowded by hyperplasia as to be thrown into low waves, thus giving the lumina an irregular outline in cross section. The connective tissue of the oviductal wall was increased in thickness and density and contained a definite stratum of smooth muscle fibers. Shell glands could not be recognized in the posterior levels of the oviducts.

The wolffian ducts and mesonephroi were not visibly affected by experimental treatment in either the females or the male.

## DISCUSSION

The response of the oviducts of the female alligators to stimulation by testosterone was an interesting and surprising result of the experiment. Evidence has been accumulating for some years that, under certain conditions, several of the male hormones may have a stimulating effect on female accessory sex structures in immature and mature mammals (Brouha and Simonnet, '28; Skowron, '34; Butenandt and Kudszus, '35; McEuen, Selye and Collip, '36; Wolff and Ginglinger, '36; Klein and Parkes, '37; Deanesley and Parkes, '37, and others). The prolonged and illuminating studies of Korenchevsky ('37) and his colleagues have led to his designation of testosterone as a 'true bisexual hormone' in that it had an approximately equal ability in both male and female castrate rats partially or completely to restore the accessory sex structures to the normal condition. Stimulation of the oviducts of the immature alligator by testosterone is in accordance with Korenchevsky's theory.

The administration of male urine extracts (Willier, Gallagher and Koch, '37) and of androsterone (Wolff, '36) to female chick embryos, and of testosterone propionate to salamander larvae (Burns, '38), resulted in inhibition of the oviducts. Willier, Rawles and Koch ('38) have reported that the administration of androsterone, dehydroandrosterone, and testosterone proprionate to female chick embryos usually brought about the regression of the left oviduct and a lengthening of the right oviduct. Since, except for the last-mentioned result (increase in right oviductal length), the administration of androgens inhibited the oviducts of female chick embryos and of salamander larvae, while testosterone stimulated the oviducts of post-hatching female alligators, it would appear that the degree of maturity of the oviduct may be one of the important factors governing the response of this structure to androgenic substances. It is readily apparent that the possible significance of other variable factors such as the nature of the experimental animal, the nature of the hormone used, and the method of hormone administration must be evaluated before final conclusions may be drawn regarding the experimental data.

The similarity in action of testosterone and of oestrone on the alligator oviduct is worthy of note. The author has shown (Forbes, '38) that prolonged injections of oestrone into immature (18 months old) alligators provoked an extreme growth of the oviducts which exceeded in its degree but not in its nature the response to testosterone of the oviducts of the females in the present experiment. In both cases there was oviductal hypertrophy associated with mucosal hyperplasia and the differentiation of a visible muscle layer.

In the light of the marked growth of the female oviduct as a result of testosterone administration, it was significant that another accessory sex structure, the clitoris, showed no gross response. That testosterone was not without effect on the male homologue of this organ was indicated by the very pronounced growth of the penis of the single experimental male. The lack of response of the clitoris of the alligator to male hormone administration is in contrast to the masculinization of the clitorides of various embryonic or prepubertal mammals as a result of testis grafting or the administration of androgenic substances (Lipschütz, '18; de Jongh and Mulder, '32; Dantchakoff, '37 b; Greene and Ivy, '37, and others). Lacassagne and Raynaud ('37) reported that daily injections of testosterone acetate into newly born mice had no effect on the penes but caused marked hypertrophy of the clitorides within 6 weeks.

The lack of response to testosterone of the mesonephroi and wolffian ducts of both male and female alligators is of interest. It has been demonstrated repeatedly in the higher vertebrates that the presence of male hormone is essential for the development of the mesonephros and its duct into an epididymis and vas deferens. Willier, Gallagher and Koch ('37) and Willier, Rawles and Koch ('38) found that the wolffian ducts of chick embryos of both sexes hypertrophied slightly following the administration of bull testis extract, while there was extreme hypertrophy of the ducts as a result of the administration of adequate quantities of extracts of human male urine and of synthetic androgens. Burns ('38) has reported that the injection of testosterone propionate in Amblystoma larvae resulted in an enormous hypertrophy of the wolffian ducts. The lack of response to stimulation of the mesonephros and mesonephric duct of the recently hatched alligator may perhaps be due to a qualitative or quantitative deficiency of the hormone employed or to an inability of these structures to respond at this age to stimulation by any male hormone. It should be noted in this connection that following the injection of whole pituitary extracts in immature (4 months old, 18 months old) alligators (Forbes, '37), there was no development of the wolffian ducts and mesonephros, although the marked testicular hypertrophy which occurred may conceivably have been associated with the elaboration of a male hormone.

## SUMMARY

A total of 10 mg. of testosterone was injected over a 91-day period into each of fourteen alligators (one male, thirteen females), 3 months old when experimental treatment began. There were nine controls (three males, six females) of the same age. The injections resulted in a uniform and striking growth and development of the female oviducts and a marked hypertrophy of the penis of the single male. The injections had no effect on the clitorides of the females and on the wolffian ducts and mesonephroi of either sex.

## ADDENDUM

Recently there have appeared two papers which should be mentioned. Kehl ('38, C. R. Soc. de Biol., T. 127, pp. 142–144) found that the injection of androsterone benzoate into adult female lizards, Uromastix acanthinurus Bell, during the period of sexual quiescence resulted in a marked activation and development of the oviductal mucosa, while the sexual segments of the kidney tubules showed a response comparable to that seen during the breeding period in male lizards. Bulliard and Ravina ('38, C. R. Soc. de Biol., T. 127, pp. 525–528) have reported that the injection of testosterone propionate into immature male and female ducks of the genus Cairina was followed by hypertrophy of both penes and clitorides.

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## PLATE 1

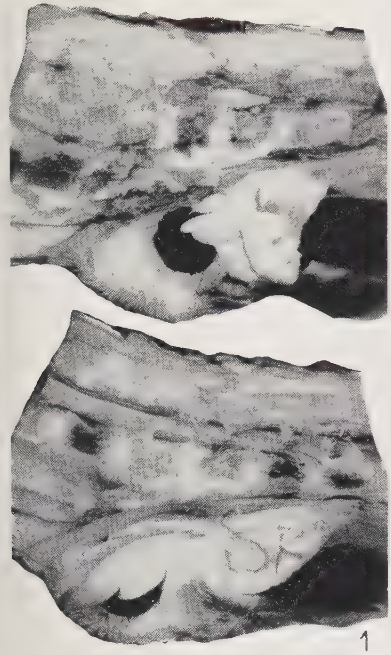
### EXPLANATION OF FIGURES

1 Median sagittal sections through the cloacal region of a control male (above) and the experimental male (below). Note the hypertrophied penis of the latter animal. × $2\frac{1}{2}$.

2 Dissections of a typical control female (left) and a typical experimental female (right). The oviducts lie just lateral to the ovaries and extend caudally to the cloaca, most of which has been resected. The oviducts of the experimental female show a marked increase in length and diameter. × $2\frac{1}{2}$.

3 A typical section through a control oviduct (upper left). The oviductal mucosa consists of a simple columnar epithelium; muscle fibers could not be detected in the wall of the duct. Part of the metanephros appears at the lower left, the mesonephros is seen at the center, and the lateral portion of the ovary is visible at the right. × 75.

4 A section through a much convoluted, anterior level of a typical experimental oviduct. Note the hyperplasia of the oviductal mucosa. A muscular stratum, not visible here, was present in the connective tissue wall of the oviduct. A part of the ovary appears at the upper left, the mesosalpinx at the right. × 75.



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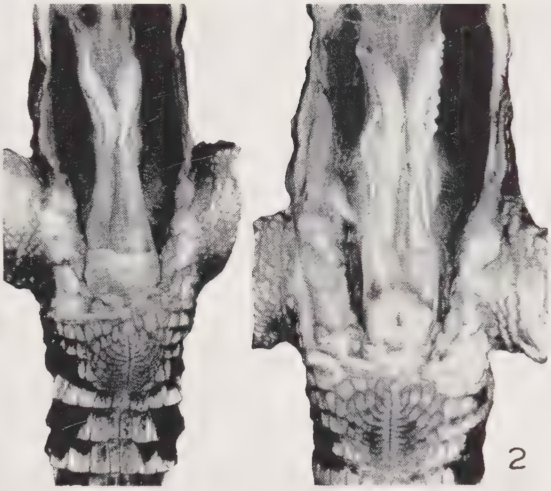

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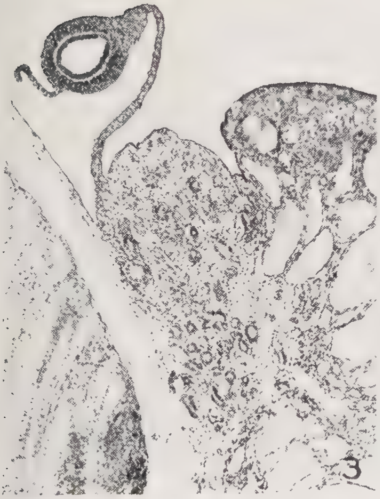

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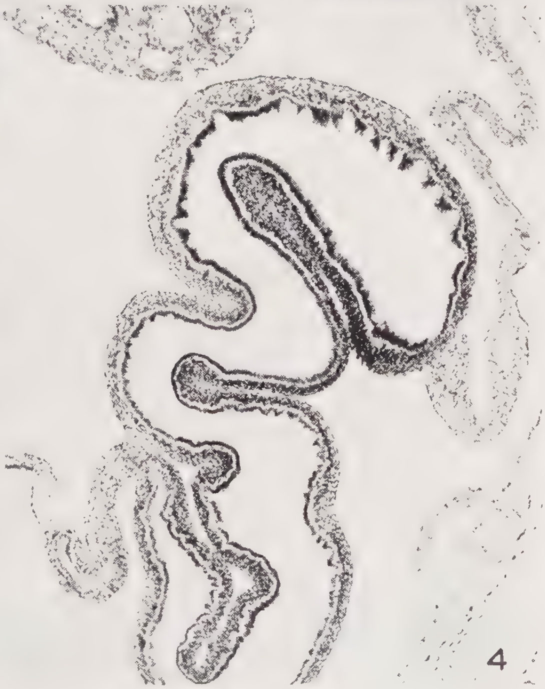

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# STUDIES ON BONE MECHANICS IN VITRO

## I. INFLUENCE OF PRESSURE ON ORIENTATION OF STRUCTURE

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TWO TEXT FIGURES AND THREE PLATES (NINE FIGURES)

### INTRODUCTION

"It is the question of the kind of functional stimulus which is effective and the manner of its action that remains as the most obscure problem in skeletogenesis" writes P. D. F. Murray ('36) in the summary of his stimulating and illuminating book on bones. The tissue culture experiments recorded below were undertaken with the object of obtaining further clues to the solution of this problem. Although the conclusions reached must necessarily be restricted to the behavior of bone tissue in culture, they may provide some indication of how this tissue reacts in the organism.

### MATERIAL AND METHODS

*Material.* Endosteal cultures derived from the tibiae of late embryonic and newly hatched chicks were used for these experiments. This material does not normally show an orientated structure and is thus very suitable for investigating the production of orientation by mechanical means. In this paper the reactions of such cultures to pressure stresses are described.

*Culture technique.* The technique of obtaining endosteal cultures is that devised by H. B. Fell ('32) who has already described it in detail. A small fragment of bone from the tibia of a late embryo or young hatched chick is explanted in a hanging drop preparation and after a few days' growth be-

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comes surrounded by a halo of emigrated cells. The original bone is then excised. The emigrated cells rapidly fill up the hole thus formed and new bone differentiates in the interior of the culture.

In order to subject the osteogenic tissue to pressure, endosteal cultures at various stages of ossification and calcification were implanted in the space between two adjacent ribs which had been explanted in vitro. The rib cultures were prepared in the following way: two adjacent ribs, with the intercostal muscles connecting them preserved intact, were isolated from a number of 17- to 21-day fowl embryos and 1- to 5-day hatched chicks. These preparations were then cut up into smaller fragments suitable for cultivation. The endosteal culture (c in fig. A) which had been grown concurrently, was placed between the ribs (AB and CD in fig. A) on the intercostal muscle (im) and the whole combination was cultivated by the hanging drop method. The medium used was composed of about 4 large drops of fowl plasma and 1 drop of concentrated extract of a 10- to 11-day fowl embryo. The cultures were transferred to fresh medium at intervals of 48 to 72 hours. During cultivation the two rib fragments were drawn together, by a process described below, and thus exerted pressure on the endosteal culture lying between them.

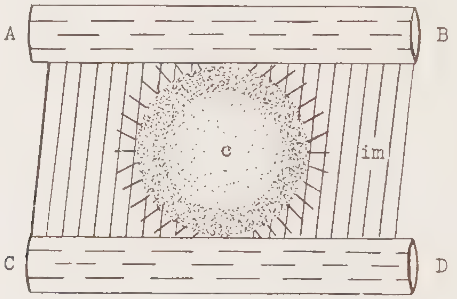

Fig. A Shows the general arrangement of the pressure experiment. The endosteal culture (c) is placed on the intercostal muscle (im) between the two ribs (AB and CD). During the culture period the ribs approach each other, thus exerting a pressure on the endosteal culture.

In all, 253 cultures were made and observed living. Of these 155 were fixed and the rest were rejected after a culture period of between 10 and 58 days. The cultures were derived from twenty-one different chicks ranging in age from the seventeenth day of incubation to the fifth day after hatching.

*Histological technique.* After different periods of growth the cultures were fixed in one of the following solutions: Zenker's or Carnoy's solution, Susa or 10% formol and were decalcified in formol-nitric acid. Serial sections were cut and stained with: haematoxylin-eosin, azan, Wilder's method (counterstained with carmalum and lightgreen), Romeis' or Weidenreich's method. Serial sections were made of 145 cultures. Of these 103 were untreated endosteal cultures, 11 were cultures of ribs and 31 were combined endosteal and rib cultures. Ten untreated endosteal cultures were fixed and stained with carmalum, haematoxylin-eosin or azan and mounted whole.

### *The structure of normal endosteal cultures*

The general behavior and development of endosteal cultures has already been described by Fell. As, however, it is important to determine the intimate structure and range of variation of untreated endosteal cultures before subjecting such material to experimental agents, a detailed description of the histological findings is given.

After the removal of the original bone fragment, usually on the sixth day of the culture period, the ring of outgrowth consisted of an inner zone composed of densely packed collagen fibers and cells, and an outer less compact region, the cells of which radiated into the culture medium. Ossification had usually already begun in the inner zone.

The form of the cells and the arrangement of the fibers depended on the degree of contraction or tension in the ring of outgrowth. If the outgrowth was subcultivated immediately after the removal of the original explant, it contracted greatly. On the other hand, if the outgrowth was left intact in the same clot after removal of the bone, the central space from which the bone was excised expanded owing to the centrifugal retraction of the clot, so that a tension was set up in the ring of outgrowth. In order to study the differences in form and structure between these two types of growth, cultures were fixed, stained and mounted whole. In the contracted state, the cells of the inner zone of the ring of outgrowth had the form of typical osteoblasts and were surrounded by loosely arranged collagenous fibers, whereas in the extended state the cells were spindle-shaped like fibroblasts and the fibers were arranged in parallel lamellae running concentrically around the central hole.

In some of both the extended and contracted cultures a fine network of fibers appeared in the central hole (fig. 1) as early as 24 hours after the excision of the bone before any cells had immigrated there. These fibers varied in thickness and were irregularly arranged. They never ended freely in the central hole, and sections showed that they were directly continuous with the osteogenic fibers of the ring of outgrowth. The fact that the fibers might appear before the cells was interesting, since the reverse usually occurred in the ring of outgrowth, the outwandering cells appearing first and the fibers later. After the central network of fibers had been formed, it became populated by cells wandering in a radial direction from the inner margin of the ring of outgrowth into the central hole. The orientation of the radially directed cells thus bore no relation to that of the chaotically distributed fibers.

The ring of outgrowth in all cultures continued to ossify and calcify, while preserving its concentric arrangement of cells and fibers. When the central hole had become filled with immigrating cells, which then lost their radial arrangement, ossification began in this region also. Cartilage often developed in the center and was directly continuous with the osteoid tissue, as described by Fell ('33). If the cultures were maintained for longer periods this cartilage became transformed into bone. The central part of the culture never showed an orientated structure either in living or fixed material (figs. 2 and 3) even after 54 days in vitro. Cells and ground substance were irregularly arranged and even the application of specific fiber stains (Wilder's, Weidenreich's method or azan) did not reveal any orientation.

### *Behavior of rib explants in vitro*

Except in certain experiments described below only the bony parts of the ribs medial to the proc. uncinatus were explanted. After a latent period, the length of which depended on the age of the chick from which the culture was derived, a few fibroblasts wandered out into the medium and at the same time the explants contracted, especially after each subculture, so that after 10 days or less the two ribs were actually in contact. The intercostal muscles between the ribs meanwhile degenerated. Any pair of bone explants, such as two metatarsals or fragments of the tibia, when placed a short distance from each other, behaved in a similar way to the ribs. Their zones of outgrowth fused and finally the two explants became enclosed in a common capsule and came in contact with each other. That the pressure exerted on a culture by the approaching ribs was quite considerable, was best illustrated by an experiment in which a fragment of scleral cartilage from a 12-day chick embryo was implanted between the ribs. The plate of cartilage was bent and actually broke within 6 days.

To investigate the tension and pressure stresses caused by the approximation of the ribs, a square hole was cut in the intercostal muscles on the third day in vitro and was filled with plasma. The explant was then transferred to fresh medium. Within 24 hours, the hole had narrowed in a direction at right angles to the ribs and elongated slightly in a direction parallel with the ribs. Cells immigrating into this space were at first always orientated parallel with the ribs; during the next 2 days the plasma in the hole liquefied and some of the cells then diverged slightly from this direction.

### *The effect of pressure on endosteal cultures*

The endosteal cultures used for these experiments were grown separately for periods ranging from 6 to 17 days though most of them were inserted between the ribs on the twelfth day. They thus differed, when first subjected to pressure, in their degree of ossification and especially of calcification which normally increases with the length of the culture period. There may be considerable variation, however, in the degree of differentiation and calcification reached by sister cultures of the same age. The intensity of calcification in a given culture is clearly indicated by the relative opacity of the ossifying zones as previously stated by Fell. It was found that cultures reacted to pressure differently according to their degree of calcification. Observations were made on forty-nine experimental cultures, some of which were drawn at intervals with the aid of a camera lucida. Owing to the thickness of the cultures it was not possible to make out details of structure, but the calcified zones and their arrangement were easily distinguishable.

*Highly ossified cultures.* On the third day after implantation between the ribs, the original ossified ring appeared slightly flattened at the site of contact with the ribs and the culture itself had become oblong with its longer axis parallel to that of the ribs. In the center a new type of structure developed, which was usually present by about the third day, but sometimes formed later. This consisted of a number of bone trabeculae orientated at right angles to the ribs. Sometimes other trabeculae similarly orientated, occurred farther away from the center, but they were shorter and tended to converge toward the center of the culture.

This type of structure which may be termed the 'resistance structure,' persisted in twelve cultures from the third to the eighth day after implantation between ribs, at which stage nine of them were fixed. During that time they prevented a further approximation of the ribs.

Histological examination of the fixed cultures confirmed and amplified the observations made on the living material. In one typical specimen (figs. 4 and 7), grown separately for 12 days and fixed 5 days after its implantation between the ribs, the main trabeculae (mt) ran at right angles to the direction of the ribs (aa) while a number of smaller, secondary trabeculae (st) converged to the center of the culture. The outermost ring of bone (cr) corresponded with the original ring of outgrowth and still showed its concentric arrangement of cells and ground substance except for the region where the main trabeculae met it. Here (bcr) the ring seemed broken and the concentric arrangement disturbed. A number of osteoclasts occurred in this region. At r in figures 4 and 7 remnants of the original bone explant were seen causing a disturbance of the resistance structure; the fragments were encapsuled by newly formed bone. Apart from a slight flattening of the side adjacent to the ribs (aa), the culture as a whole showed comparatively little compression.

*Slightly ossified cultures.* Fifteen cultures at an early stage of ossification were implanted between ribs, six of which were fixed and sectioned after a further period of 5 to 12 days. Such implants behaved differently from well-ossified cultures similarly treated. On the third day after being placed between the ribs, the cultures had become much elongated in a direction parallel with the ribs, and the concentric ring broke open in the same direction. In the interior calcified trabeculae appeared running parallel with the ribs. This type of structure may be called 'compression structure.' After this stage was reached, further approximation of the two ribs was usually prevented by the now calcified implant, but sometimes, where calcification was less intense, the ribs continued to approach each other for a few more days.

Figures 5 and 8 show the histological appearance of a culture with a typical 'compression structure.' This specimen was implanted between ribs on its sixteenth day in vitro and was fixed after a further 12-day growth. It was greatly flattened in a direction at right angles to that of the ribs (aa), and the cells and ground substance of its center (mt) were orientated more or less parallel with the ribs. The original osteoid ring (cr) was broken at both ends (bcr) so that even here the bone substance was arranged parallel with the ribs. The presence of large numbers of osteoclasts (o) filled with bone débris indicated that extensive resorption and reconstruction were in progress.

If the cultures were rather more calcified at the time of implantation than those described above, a structure intermediate between the 'resistance' and 'compression' types developed. Eighteen of these intermediate cultures were observed in vitro; twelve of which were fixed 4 to 8 days after their implantation between the two ribs. In such cultures small trabeculae had formed by the third day, which at first were orientated at right angles to the ribs. During the next few days they increased in thickness, but at the same time shifted into a position nearly parallel with the ribs. This shifting was brought about mostly by deposition of bone parallel to the ribs. At the same time the general outline of the culture became more flattened.

Sections of cultures with the intermediate type of structure (figs. 6 and 9) showed very clearly the oblique trabeculae (mt), described above, which had been shifted by the pressure until they lay at an acute angle to the direction of the ribs (aa). Where they united with the ring (cr) formed by the original outgrowth a break and disturbance of the concentric arrangement occurred in the ring (bcr). Here a large number of osteoclasts was found. In the implanted cultures the degree of flattening of the general outline was correlated with the nature of the internal structure and the nearer this approached the resistance type, the less the flattening produced (compare figs. 4, 5 and 6).

In transverse sections made at right angles to the plane of the culture cover slip, it was seen in cultures of the intermediate type that the superficial bone adjacent to the intercostal muscles had a compression structure with cells and fibers orientated parallel with the ribs, while bone near the other surface, where pressure was less, often had a resistance structure. The main trabeculae, on the other hand, being situated in the middle of the culture, had a uniform structure throughout their thickness. As in cultures of the resistance type, cartilage often occurred in the spaces between the main and the secondary trabeculae in the intermediate cultures, whereas cartilage was never observed in implants of the compression type.

*Control implants.* To find whether implantation between ribs produced any effects other than those due to pressure, cultures were made of the lateral region of the ribs including the proc. uncinatus. The processus was fixed between the ribs so as to prevent their approximation, and an endosteal culture was then inserted into the intercostal space. The distance between the ribs remained constant throughout the experiment, although the muscle fibers degenerated in the usual way. Thus pressure was the only factor present in the previous experiments which was excluded from this. Four cultures fixed 7 days after implantation under these conditions showed no difference in structure from similar cultures grown separately, i.e., without being implanted on rib cultures, for the same period.

## DISCUSSION

Some interesting points arise from the experiments in which the zone of outgrowth of endosteal cultures was subjected to tension by removing the original bone and allowing the retracting clot to pull on the ring of outgrowth. The results showed that the form of osteoblasts depends on the tension to which they are subjected. They also showed that, although osteoblasts and osteogenic fibers are orientated parallel to each other when tension is exerted on the tissue, in the absence of tension the arrangement and formation of fibers are independent not only of the arrangement but even of the immediate proximity of the cells. This was clearly demonstrated by the fact that osteogenic fibers with a chaotic distribution appeared in the central hole of the endosteal culture before the radially migrating cells had populated this region.

No orientation whatever could be distinguished in the control material of the 204 untreated endosteal cultures studied. On the other hand, the forty-nine cultures subjected to pressure all showed a definite orientation of structure. Two main types of orientation could be distinguished: a) along the lines of tension (compression structure), and b) along the lines of pressure (resistance structure). As described above, the degree of calcification of the culture when first subjected to pressure determined which type of structure should develop. Any soft material gives way to pressure along the lines of tension, whereas hard material breaks in the line of pressure. Thus in slightly calcified cultures the original ring of bone broke along the lines of tension and provided an outlet for the central material in this direction. This caused orientation along the lines of tension and the formation of the compression structure.

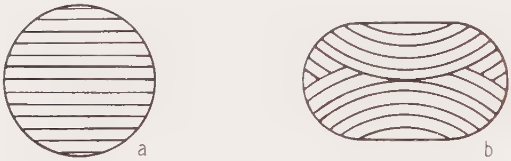

Fig. B Illustrates the change of pattern impressed on the surface of a paraffin disc, brought about by pressure. The paraffin disc is surrounded by a ring of vulcanized fiber. When subjected to the pressure of a vice the ring breaks in the line of pressure and pattern (a) of the paraffin disc changes to pattern (b).

In highly ossified cultures, however, no such outlet was possible as the concentric ring broke if it broke at all in the lines of pressure. Here the ribs by their pressure pushed the material into the central region which representing the site of the excised original bone explant, was at first the softest and least dense part of the culture. Thus a condensation of material in the lines of pressure resulted.

Experiments on mechanical models illustrate this point. A disc of paraffin or plasticine surrounded by a thin brass ring is transformed by pressure into an elliptic body. A pattern of concentric circles impressed on the surface of the paraffin is transformed into a system of ellipses, the long axes of which run along the lines of tension. Under higher pressure the brass ring breaks along the lines of tension.

A ring of vulcanized fiber breaks under pressure along the lines of pressure. If this ring is filled by a disc of paraffin on which a pattern of straight lines parallel to the lines of tension is impressed, and the whole arrangement subjected to pressure from a vice, the hard outer ring breaks along the lines of pressure. The pattern on the surface of the paraffin is changed into two families of curves with their convex sides opposing and having the line of centers along the direction of the lines of pressure (fig. B, a and b). This model shows that the derangement of material is greatest in the lines of pressure, while in the first model the greater derangement occurs along the lines of tension.

Thus the formation of both types of structures is explained in a mechanical way. But the presence of osteoclasts indicates that the process is not entirely passive as far as the bone tissue is concerned but is associated with an active remodelling of structure. Although these in vitro experiments show conclusively that bone tissue can react directly to mechanical stresses, they do not exclude the possibility, as Murray pointed out, that in the organism the response of the bone may be indirect.

I have pleasure in acknowledging my indebtedness to Dr. H. B. Fell for her helpful suggestions, critical advice and the interest she showed throughout this work; to the British Fund for German Jewry for a maintenance grant; to Mr. V. C. Norfield for the photomicrographs.

## SUMMARY

1. Orientated structures were produced in unorientated bone tissue in vitro, by subjecting endosteal cultures to suitable pressure.

2. The type of structure produced, whether of the resistance, compression or intermediate type, depended on the degree of calcification of the tissue at the time when the pressure was applied.

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## DESCRIPTION OF PLATES

All the figures are photomicrographs. Figures 3, 7, 8 and 9 are enlargements of areas outlined in white in figures 2, 4, 5 and 6, respectively. All the sections of cultures illustrated were cut parallel with the plane of the cover slip of the original culture vessel.

## LIST OF ABBREVIATIONS

aa, direction of rib AB in figure A
bcr, break in the concentric arrangement of the ring of outgrowth
cr, original ring of outgrowth
mt, main trabeculae
o, osteoclasts
r, remnants of original bone explant
st, secondary trabeculae

## PLATE 1

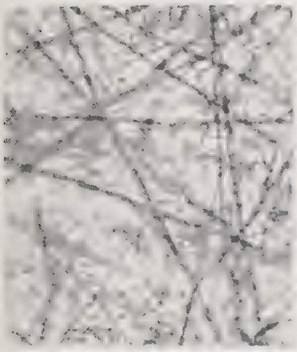
### EXPLANATION OF FIGURES

1 Part of section through an endosteal culture fixed 24 hours after the excision of the original bone explant. In the central hole a fine network of osteogenic fibers has appeared before any cells had immigrated there. (Romeis' method). × 590.

2 Section through an untreated endosteal culture fixed 16 days after the excision of the original bone explant. The ring of original outgrowth (cr) shows a concentric arrangement of cells and fibers while no orientated structure can be detected in the center of the culture. (Weidenreich's method). × 72.

3 Enlargement of area outlined in white in figure 2. × 144.

4 Section through an endosteal culture fixed 5 days after its implantation between the ribs. Resistance structure. The main trabeculae (mt) run at right angles to the direction of the ribs (aa). The outermost ring of bone (cr) is broken in the region where it is met by the main trabeculae. Remnants of the original bone explant (r) cause a disturbance of the resistance structure. (Azan.) × 155.



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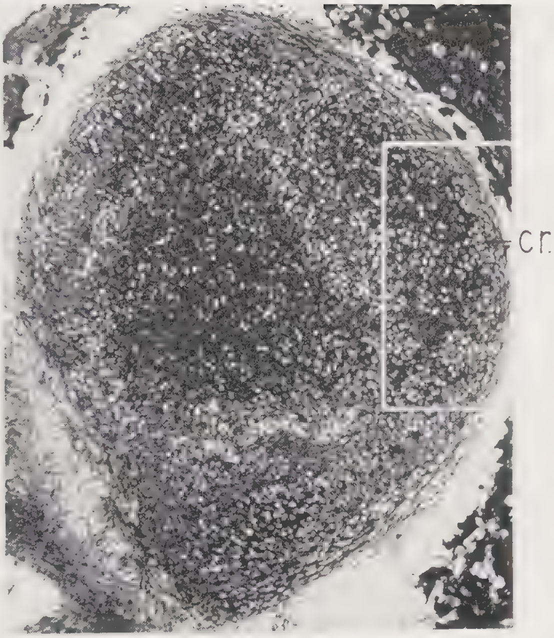

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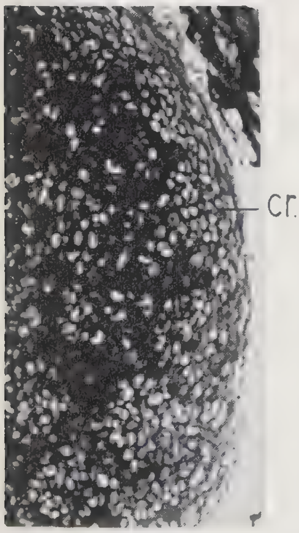

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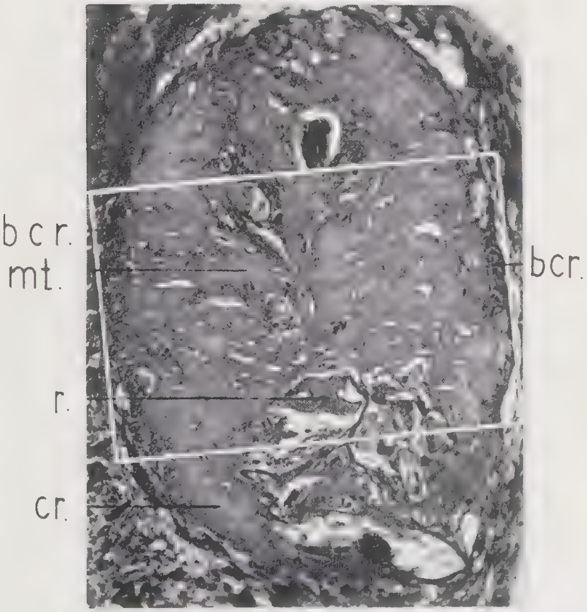

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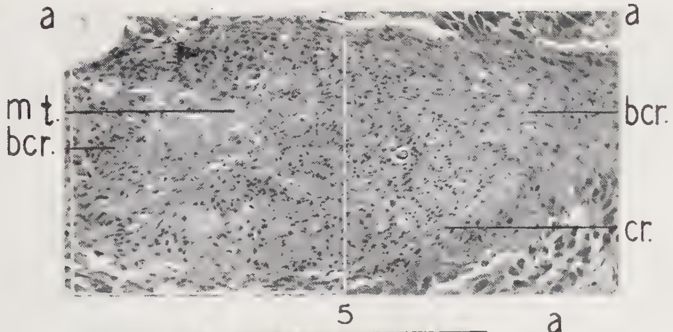
# PLATE 2

## EXPLANATION OF FIGURES

5 Section of endosteal culture fixed 12 days after implantation between ribs. Compression structure. The main trabeculae (mt) run parallel with the direction of the ribs (aa). The original osteoid ring (cr) is broken at both ends (bcr). (Haematoxylin-eosin). × 130.

6 Section of endosteal culture fixed 4 days after implantation between ribs. Intermediate structure. The main trabeculae (mt) run in an oblique direction to the ribs (aa). Where they meet the ring (cr) formed by the original outgrowth, a break (bcr) and disturbance of the concentric arrangement is seen. (Azan.) × 200.

7 Enlargement of area outlined in white in figure 4. Secondary trabeculae converge to the center of the culture. (Compare figure B, b). × 310.



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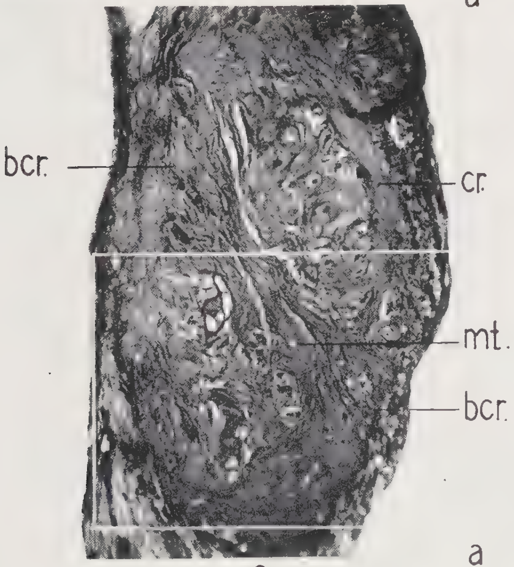

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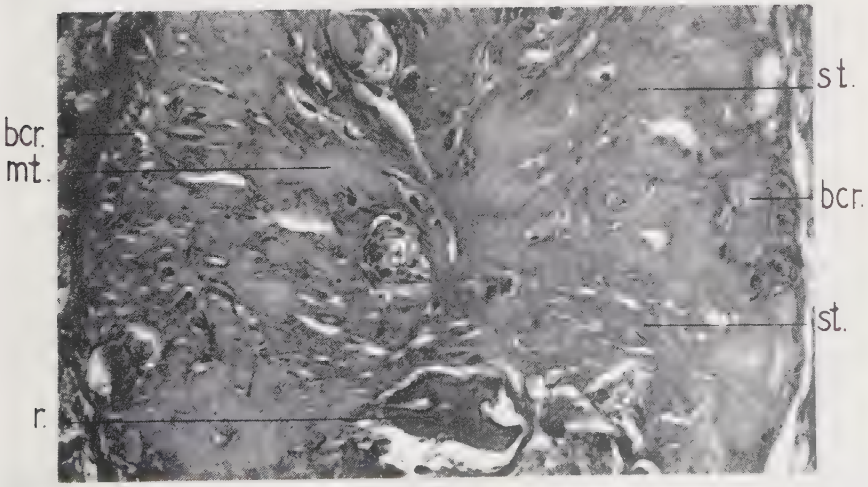

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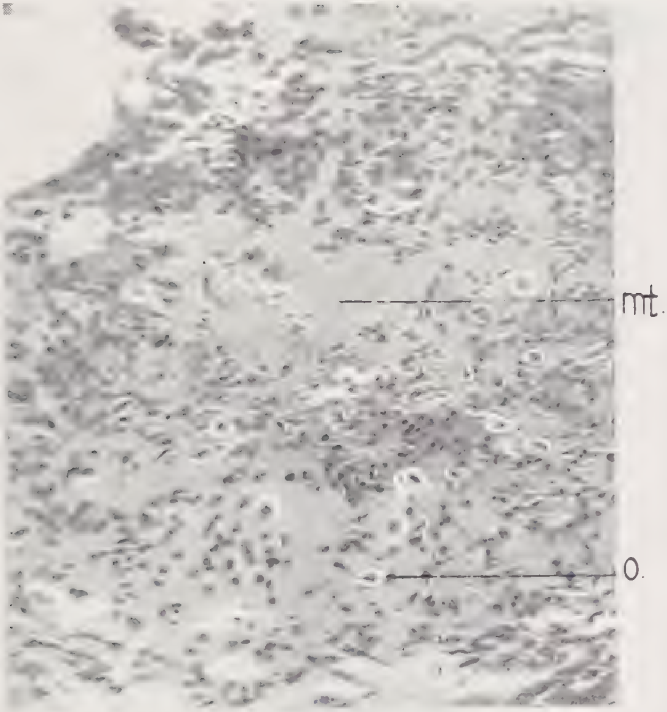
## PLATE 3

### EXPLANATION OF FIGURES

8 Enlargement of area outlined in white in figure 5. Note the large number of osteoclasts (o). × 260.

9 Enlargement of area outlined in white in figure 6. × 400.



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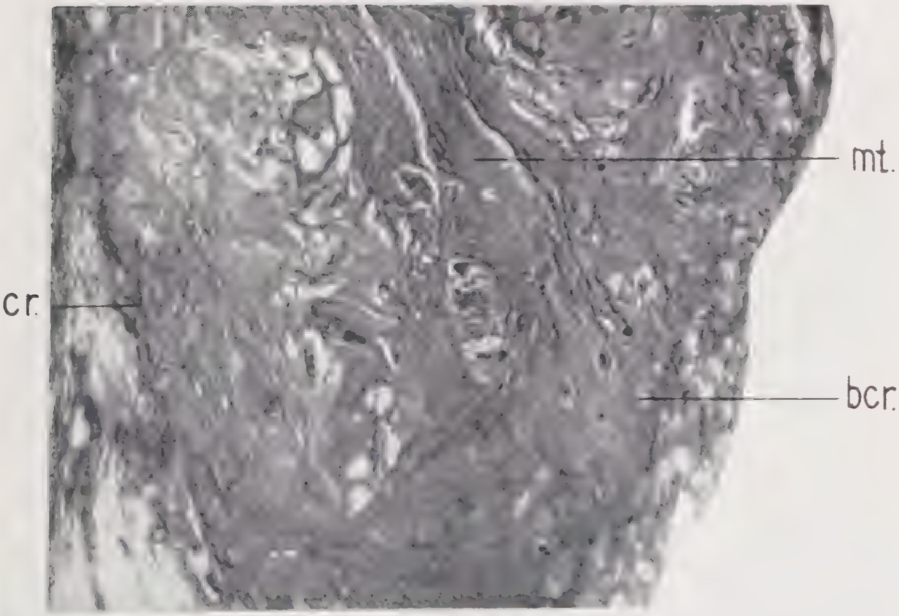

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# THE BODIAN TECHNIC AS APPLIED TO THE COMPLICATED CELL BOUNDARIES OF THE RENAL EPITHELIUM IN MAMMALS

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TWO FIGURES

As is well known, the shape of the cells in various portions of the renal tubules in mammals is frequently quite complicated (for a broad statement of the problem see Zimmermann, '11). The demonstration of the highly irregular cell boundaries can usually be accomplished, and then with but partial success, only by specialized technics, which are rather limited in number. The object of this note is to add to this number the technic recently developed by Bodian ('36, '37) for the study of the nervous system. In our hands this method likewise has given only patchy impregnation of the cell boundaries, and none of the variations in the technic which we have employed has yielded impregnation of more than a small minority of the tubules visible in a given section. Nevertheless, when successful results are obtained the pictures are so sharp and so instructive that the method is to be highly recommended as supplementary to those already available. The technic furthermore has the distinct advantage of being applicable to material well fixed by perfusion with any fixative, embedded in paraffin, and sectioned at any desired thickness.

Figure 1 illustrates the appearance, in a Bodian preparation,[2] of the cell boundaries in the pars convoluta of the proxi-

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[1] Fellow of the Henry Strong Denison Medical Foundation.

[2] Cat killed with illuminating gas; perfused via thoracic aorta with salt solution, followed by 'Susa'; small blocks of cortex (apparently well perfused) then immersed in 'Susa' for a short time before dehydration, etc. Preparation in protargol 24 hours.

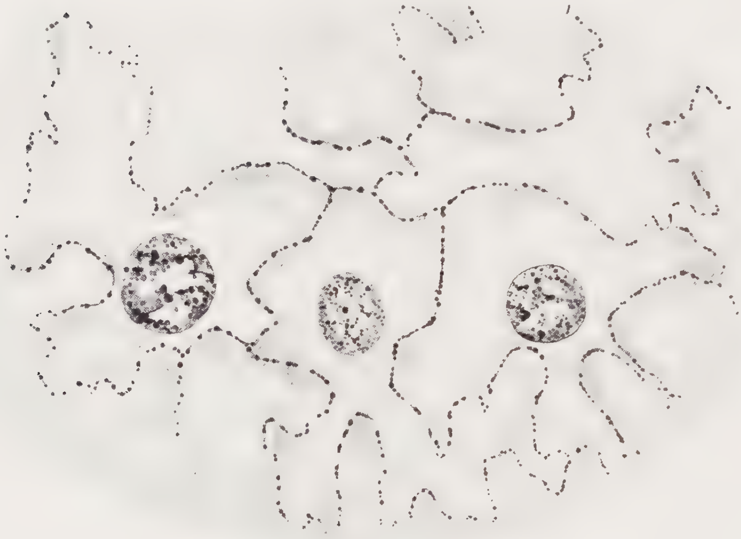

Fig. 1 Impregnation of cell boundaries in pars convoluta of proximal tubule of cat nephron. Bodian preparation; paraffin section, 5 μ. Camera lucida drawing at × 3150; as reproduced, × 2520.

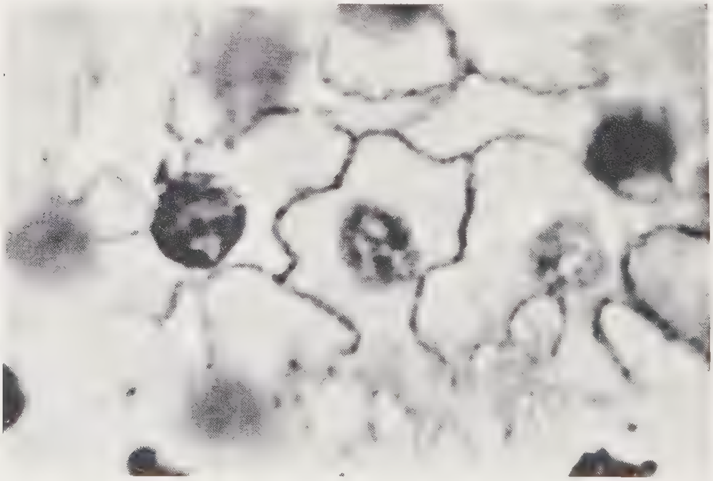

Fig. 2 Photograph of microscopic field illustrated in figure 1. The granular character of the impregnation is well shown. The greater intensity and thickness of some of the lines as compared with the drawing is the result of superposition of granules at different levels. In the drawing this has been obviated by reproducing the appearance at a single sharp focus.

mal tubule of the cat nephron. The figure represents a selected instance, showing the maximal impregnation which we have been able to obtain. In many instances the picture is more delicate, often exceedingly so, but is always of the same essential character. The drawing illustrates a section through the renal epithelium at a point where the tubule is undergoing a rather sharp bend, so that the cells are transected at varying levels between the lumen and the basement membrane. In the peripheral portion of the drawing, particularly to the left and below, as the cell boundaries approach the basement membrane the impregnation is more delicate than in the central portion. This increase in delicacy toward the basement membrane is a rather characteristic finding.

The cell boundaries are impregnated as rows of unevenly spaced black dots. That these dots do not represent filamentous structures, seen in cross section, is obvious from the fact that (in this and other preparations) the boundaries maintain the same dotted character irrespective of the plane in which they are cut. Furthermore, in places where one can study the sides of the cells in surface view, one finds sheets of dots, spaced with variable regularity, the interstices between the dots being apparently entirely unimpregnated. At the luminal surface of the cells the boundaries (terminal bars) are likewise dotted.

The vacuolation of the cytoplasm is an expression of the fat droplets originally present in the cells. The delimitation of these vacuolar spaces is in some instances sharper in the original preparation than in the drawing. As has been amply demonstrated, the cells of this portion of the cat nephron are usually laden with fat under normal conditions, whereas the terminal portion of the proximal tubule is fat-free (Peter, '09; Zimmermann, '11; Foote, '36).

## SUMMARY

The Bodian technic for nervous tissues can be used successfully for the impregnation of the complicated cell boundaries of the renal epithelium in mammals. The impregnation is exceedingly sharp, and is granular in character.

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# DISTRIBUTION OF VITAMIN C IN THE ADRENAL GLAND OF THE MOUSE WITH REFERENCE TO THE NATURE OF THE X ZONE

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TWO TEXT FIGURES AND ONE PLATE (FOUR FIGURES)

For the detection of vitamin C (ascorbic acid) in tissues, a silver nitrate reagent is used. This reagent stains the adrenal cortex markedly, but produces little or no reaction in the medulla (Szent-Györgyi, '28; Giroud and Leblond, '34). In regard to this reaction, only brief reports of the adrenal gland of the mouse, without mention of the X zone, have been given (Bourne, '33; Leblond, '34).

The X zone in the adrenal of the mouse (fig. 1) has been studied extensively in various normal and experimental conditions. In the male, it disappears at puberty (Whitehead, '33 a) and reappears following castration. The X zone of the castrated male, however, involutes either slowly in older castrated animals (Howard, '30) or rapidly after injection of various male hormones (Deanesly and Parkes, '37; Cramer and Horning, '37). In the female mouse, the large X zone of the immature animals involutes rapidly toward the end of the first pregnancy, or slowly when no pregnancy occurs (Howard-Miller, '27; Deanesly, '28).

There is disagreement as to the exact nature of the X zone. Cramer ('28) and Cramer and Horning ('37) consider it as part of the medulla, but most authors think it belongs to the cortex (Howard-Miller, '27; Deanesly, '28, '38; Whitehead, '33 a). In this investigation the silver nitrate reaction, specific for the adrenal cortex, was used in order to determine the cortical or medullary nature of the X zone. In a preliminary report, it was noted that the X zone of the virgin female mouse reduces silver nitrate as does the true cortex (Leblond and Gardner, '38). However, since the X zone of castrated and immature males is not necessarily of the same nature as the X zone in virgin females our observations were continued and extended to males.

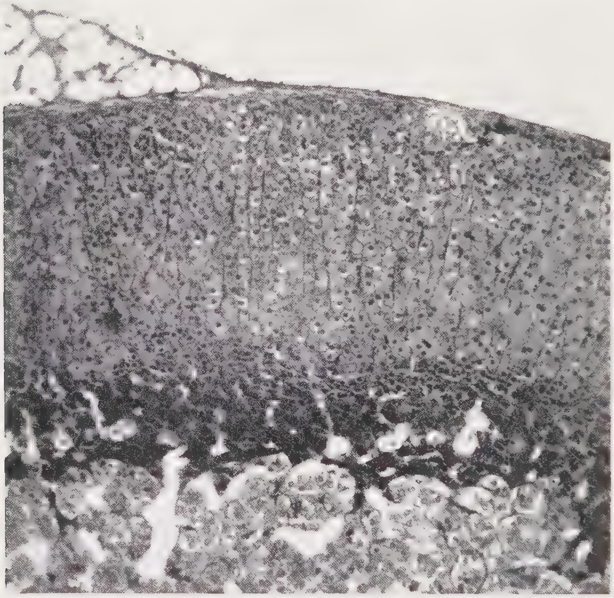

Fig. 1 An adrenal of virgin female. The X zone is prominent. Hematoxylin eosin. × 85.

## METHOD

The mice[1] were fed a diet without additional vitamin C (Purina Fox Chow, in part).

After the mice were killed by decapitation, silver nitrate was injected into the aorta through a blunted paraffined needle.

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[1] We are indebted to Doctor Dobrovolskaīa-Zavadskaīa and Doctor Strong for the mice of pure strains used in this experiment. Some of the strains used have been described by Dobrovolskaīa-Zavadskaīa ('37) and Strong ('35).

In order to wash the blood vessels 2 to 4 cc. of 50% levulose were first injected. Then 10 to 20 cc. of a 10% silver nitrate with 1% acetic acid were injected, all operations being performed very rapidly.

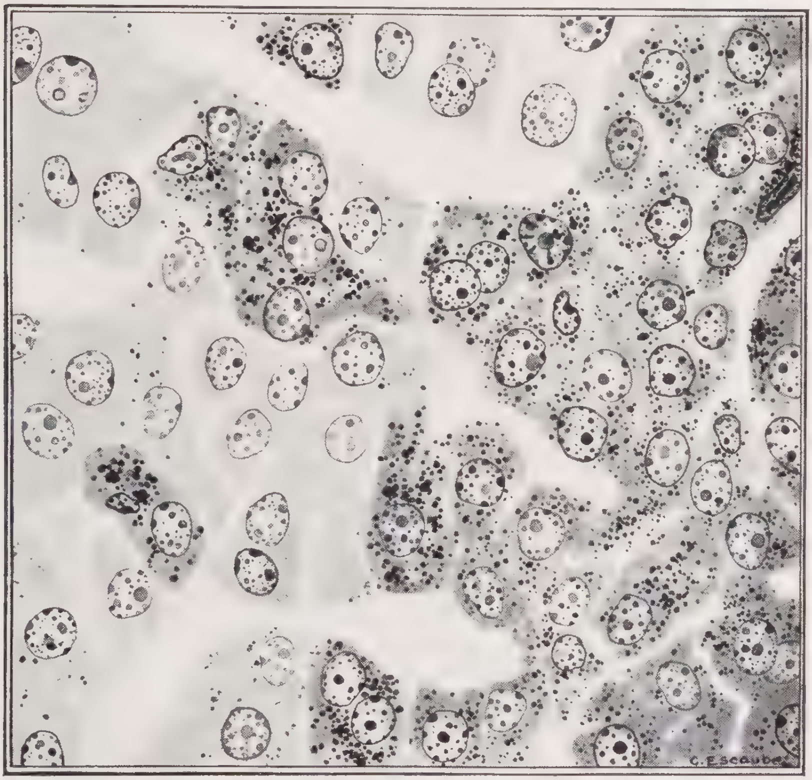

Fig. 2 Camera lucida drawing of the limit between the X zone and the medulla. The cells taking silver nitrate are more darkly stained by common dyes, as are the X zone cells. Acid silver nitrate and hematoxylin eosin.

A dim light must be used, while preparing sections of the tissue according to the usual paraffin method. The slides, after removal of the paraffin are treated with sodium hyposulfite. A few sections from the middle of the ribbon were then stained lightly with hematoxylin and eosin. Most of the sections were not stained. The details of the technique will be found elsewhere (Leblond, '34; Giroud and Leblond, '35, '36).

The examination of the slides, frequently showed an irregular penetration of the reagent. The specificity of the reaction has been previously discussed (Giroud and Leblond, '36, '37; Lison, '36).

TABLE 1

| | *Strain* | *Number of animals* | *Age (in days)* | *Extent of development of X zone* | *Intensity of the silver nitrate reaction* | | |
|---|---|---|---|---|---|---|---|
| | | | | | Fascicular zone | X zone | Medulla |
| Immature males | C3H [1] | 3 | 20 | + | +++ | +++ | — |
| Normal adult males | JK [1]<br>C3H [1] | 6 | About 90 | 0 | +++ | | — |
| 6-month castrated adult males | CHI [1] | 3 | 263 | + | +++ | + | — |
| Male hormone treated for 2 weeks (♂) | N [1] | 1 | ? | 0 | +++ | | — |
| Virgin females | 17 [2] | 6 | 23–30 | +++ | +++ | +++ | — |
| | 17 [2] | 3 | 60 | ++ | +++ | +++ | — |
| Pregnant females (middle of first pregnancy) | 17 [2] | 3 | 66, 66, 70 | ++ | +++ | +++ | — |

[1] Strong's ('35) inbred strains.

[2] Dobrovolskaīa-Zavadskaīa's ('34) strain.

## RESULTS

In all animals the fascicular and reticular zones of the cortex gave a marked reaction for ascorbic acid (table 1). None was observed in the glomerular zone (fig. 4). In the fascicular and reticular zones, silver granules filled the cells with the exception of the nucleus. The granules were of various sizes, usually moulding themselves to the mitochondria, and like these, disposed between the lipoid droplets in the cytoplasm. On the contrary, no or only rare granules were observed in the cells of the medulla (fig. 3). Some silver precipitated along the walls of the blood vessels in this part of the gland.

The large X zone in the adrenals of virgin females (fig. 3) and the smaller ones in the glands of immature males [2] reduced the silver nitrate in a similar way. The cells were filled with silver granules, which, apparently because of the absence of lipoid granules, were usually less regularly disposed and sometimes coarser than in the fascicular zone. In any case, such a marked reaction is of a 'cortex type.' The boundary between medullary and X zone cells was distinct (figs. 5 and 6). X zone cells isolated in the medulla were easily detected with silver nitrate (figs. 5 and 2). Their nature was confirmed, afterward, by overstaining with hematoxylin and eosin (fig. 2).

The castrated animals were examined 6 months after castration. Very little typical X zone was left in two of them. This reduced silver nitrate as did the active parts of the cortex. Most of the remnants of the X zone were formed of flattened connective tissue-like cells containing little cytoplasm and a few silver granules.

## DISCUSSION

The distribution of ascorbic acid (as detected by the silver nitrate reaction) in the adrenal of the mouse, was similar to that found in other mammals. The reaction was marked in the cortex, with the exception of the glomerular zone, and was minimal in the medulla. Reactions of the Golgi apparatus, as found in other species, were not found in the cells of the medulla.

It is likely that, as has been shown in other species, the absence of reaction in the adrenal medulla does not imply the absence of ascorbic acid; some ascorbic acid is present, but inhibiting factors hinder its ability to reduce silver nitrate. In the cortex, where a marked reaction was found, the reducing properties of ascorbic acid are not inhibited ('active ascorbic acid,' Giroud and Leblond, '36).[3]

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[2] In the immature male, Deanesly ('38) does not identify an X zone.

[3] In the four endocrine glands where the silver nitrate reaction is present, one observes this reaction only in the secreting cells. The inactive cells, such as those in the glomerular zone of the adrenal, do not react (Giroud and Leblond, '36). The fact that the X zone gives a marked reaction suggests that this is an actively secreting zone, and the poor reaction of involuting X zones of castrates suggests their lack of secretory activity.

The marked reaction found in the X zone indicates the presence of active ascorbic acid. The similarity of the reaction in X zones of male and female mice suggests no sex differences.

As to the cortical or medullary nature of the X zone the disagreement, between Cramer and other workers, has been stated above. The reticular arrangement of the cells, different from the pseudo-acinar groups of cells in the medulla, and the absence of chromaffinity[4] are in favor of the 'cortical' nature of the X zone. Also, Deanesly ('28, '38), emphasizes that the X zone is connected with adrenal growth, since it develops only "when the whole cortex is growing actively, as for example in normal prepubertal females or in males after castration." The observation by Whitehead ('33 b) that, when the X zone develops, lipoids are deposited in the cortex, and inversely also link X zone and cortex.

Silver nitrate staining permits the differentiation of cortical and medullary tissues. The interrenal body of selacians (Szent-Györgyi, '28), and the interrenal cords of birds (Giroud and Leblond, '35) reduce silver nitrate as well as the mammalian cortex. Adrenal medulla, and corresponding structures of inferior vertebrates do not reduce it. In the embryo also, before cortical and medullary cords are separated, silver nitrate permits one to recognize the nature of each cell, ibid, '35). A positive reaction thus appears quite specific of the adrenal cortex. Such a reaction in the X zone compels one to consider this zone as a part of the cortex.[5] More precisely it is a well-developed reticular zone. The X zone being made of true cortical tissue, appears as a formation which in the mouse is associated with the hyperdevelopment of the cortex occurring in diverse physiological conditions.

[4] Observed after prolonged fixation with bichromate. See also Cramer and Horning ('37).

[5] In the body, reactions comparable to that of the cortex and X zone were also found in the hypophysis, corpus luteum and interstitial cells of the gonads. The possibility that the X zone might correspond to one of these tissues has not been eliminated, but is unlikely in view of the results of Deanesly and Whitehead linking the cortex and the X zone physiologically.

If the X zone behaves like true cortex, then it should not be directly dependent on gonadal hormones, as the relations of the X zone with puberty, pregnancy castration and male hormone injections suggested at first.[6] Indeed the maintenance of the X zone by estrin (Burrows, '36) could not be confirmed by Lacassagne and Raynaud ('37). Also the formation of an X zone in the absence of male hormone (as before puberty or after castration) is not a direct relationship, since when male hormone secretion was suppressed by hypophysectomy of males, an X zone was not observed to form in the adrenals (Leblond and Nelson, '37). Similarly in the case of pituitary deficiency, such as in dwarf mice, castration did not determine the appearance of an X zone (Deanesly, '38). Therefore, the action of male hormone on the X zone of the male, as probably that of pregnancy in the female, works through the hypophysis.

The necessity of the hypophysis for the maintenance of the X zone has been proved by the degeneration of the reticular and X zones after hypophysectomy in virgin and lactating females (Leblond and Nelson, '37). The reasons put forth above to look upon the X zone as true cortical tissue make it probable that the hypophyseal factor involved is the adrenotropic hormone. For final decision on this point, further experiments are necessary.

## SUMMARY

1. The acid silver nitrate reaction for vitamin C was applied to the adrenal of the mouse. No reaction was found in the medulla. A positive reaction was observed in the cortex, with the exception of the glomerular zone.

2. A marked reaction of cortical type was found in the X zones of virgin females, immature males and, to a less extent, old castrated males.

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[6] Grollman ('37) attributed to the X zone the well-known androgenic activity of the adrenal, which, however, is present in species without X zones (Hodler, '36; Reichstein, '36; Davidson, '37). The androgenic capacity seems a general property of cortical tissue, and as such, is shared by the X zone.

3. The specificity of the silver nitrate reaction toward cortical tissue permits one to conclude that the X zone is true cortical tissue, probably a hyperdevelopment of the reticular zone.

Thanks must be expressed to Dr. A. Lacassagne for his general help and advice in the course of this investigation.

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# PLATE 1

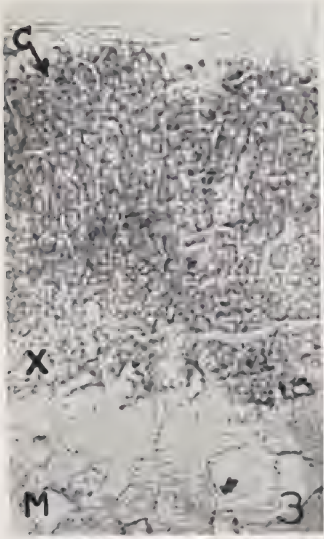
## EXPLANATION OF FIGURES

3 A vitamin C reaction on the adrenal of a 60-day-old female. The true cortex (C) and the X zone (X) show a marked reaction and cannot be distinguished. Below, the medulla (M) is not stained. Acid silver nitrate. × 160.

4 Ibid. Contrast between the absence of reaction in the glomerular zone and the marked reaction in the fascicular zone. Acid silver nitrate. × 600.

5 Ibid. Limit between the X zone (X) and the medulla (M). The medulla, where blackened red blood corpuscles testify to the penetration of the reagent, is not stained. The X zone shows identations (right hand side). A group of X zone cells are observed in the medulla (left hand side). Acid silver nitrate. × 600.

6 An adrenal of an immature male. Limit between the X zone and the medulla. Acid silver nitrate and hematoxylin eosin. × 600.



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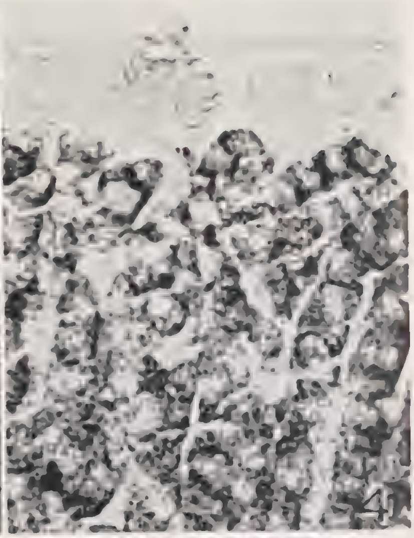
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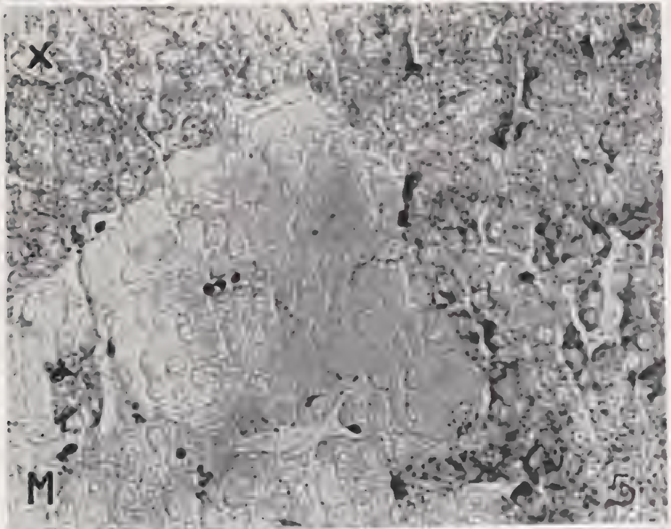

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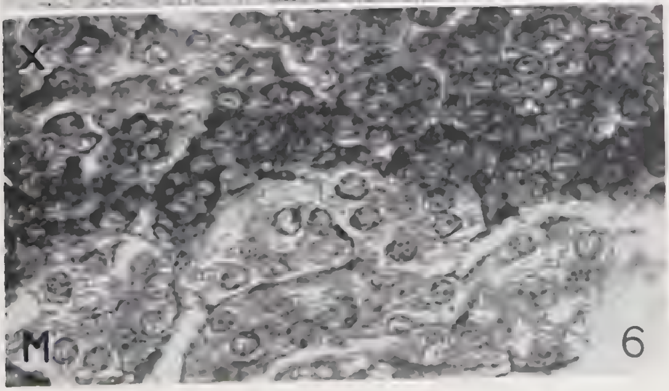

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# SOLUBILITY STUDIES OF THE SECRETION GRANULES OF THE GUINEA PIG PANCREAS

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Woerner ('38) has described, for the first time, degenerative changes in the alpha cells of the island of Langerhans, following continuous intravenous injection of dextrose in guinea pigs. These changes occurred when moderate amounts of sugar were injected and there was no resulting extensive hyperglycemia or glycosuria. However, these animals exhibited evidences of increased metabolic rate with extensive loss of weight and there were coincident fatty changes in the mitochondria of the acinar cells of the pancreas.

It occurred to us on the basis of these findings that there might be some relation between exhaustion of the alpha cells and increased fat metabolism under conditions of increased oxidation of sugar. In order to test this hypothesis it seemed desirable to obtain an extract of the alpha cells. Consequently the following solubility studies were undertaken.

I. The pancreases from three guinea pigs, whose pancreatic ducts had been ligated for 3 weeks, were frozen in liquid isopentane and dehydrated in vacuo at −40°C. Pieces of this tissue were treated with twelve organic and nine aqueous solvents and one piece was put directly into formol-Zenker for a control. The organic solvents used were: methyl alcohol, ethyl alcohol, butyl alcohol, iso-butyl alcohol, propyl alcohol, iso-propyl alcohol, iso-amyl alcohol, allyl alcohol, ethylene dichloride, ethylene glycol, methyl cellosolve, and butyl cellosolve.

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The tissue in the organic solvents was subjected to negative pressure, to extract the air from the tissue, and allowed to remain in the solvent at room temperature for 24 hours. The pieces were then cleared in oil of bergamot, embedded in paraffin and sectoned at 5 to 8 μ. One series of sections was mordanted with 1% osmic acid and stained with aniline acid fuchsin and methyl green; another was mordanted with 2.5% aqueous solution of potassium bichromate and stained with neutral gentian.

The aqueous solvents used were: distilled water, 0.85% sodium chloride, 10% sodium chloride, 0.1% acetic acid, 0.1% sodium hydroxide, 2.5% glycerine, 5% dextrose, 10% sucharose and 1% urea. The pieces of tissue were put into the aqueous solvents at room temperature for 1 hour. They and the control piece were then transferred to formol-Zenker, subjected to negative pressure, and then left in the fixative for 24 hours. Subsequently the tissues were washed, dehydrated, cleared, embedded in paraffin, sectioned and stained by the two methods used for the tissues treated with organic solvents.

With freezing and drying in vacuo, the restricted permeabilities of the cell membranes are abolished and solvents can readily penetrate the cell. Moreover, the solvents are not diluted by water in the cell. By this method, then, the action of the pure solvent on the intracellular structures can be determined directly.

The action of these solvents may be briefly summarized:

1. The pure organic solvents used, with the exception of ethylene glycol, modified but did not remove the alpha, beta or zymogen granules. Ethylene glycol removed them all.

2. Of the aqueous solvents, distilled water, normal salt solution, 10% sodium chloride and 5% dextrose removed the alpha granules; 0.1% sodium hydroxide removed both alpha and beta granules; 1% urea modified both alpha and beta granules; 5% dextrose modified the beta granules.

Since the zymogen granules may almost completely disappear following ligation of the duct, the absence of zymogen granules in the aqueous series could not be interpreted as solution of them by the solvent.

II. Whole fresh guinea pig pancreas was extracted for 24 hours at refrigeration temperature 1) with distilled water, 2) with normal salt solution and 3) first with an equal volume of 95% alcohol for 12 hours and then with distilled water for 24 hours. Pieces of each were then fixed for 24 hours in formol-Zenker, washed, dehydrated, cleared, embedded in paraffin, sectioned and stained with aniline acid fuchsin, methyl green and with neutral gentian.

Examination of the sections revealed: A) The alpha granules were removed with both water and normal salt solution. B) The beta, zymogen and Mankowski granules and even the mitochondria were resistant to both. C) After extraction first with alcohol and then with water, only the Mankowski granules remained. From this we may deduce that the alpha granules treated with alcohol, to which they are resistant, may be subsequently removed by water.

III. The reaction of pieces of fresh pancreas to various aqueous solvents was observed directly under the oil immersion lens of the microscope.

The identification of the zymogenic and Mankowski cells in fresh tissue is easy with the dark-adapted eye, because of the size, refraction and distribution of the characteristic granules. The zymogen granules are the largest, most highly refractile and occur grouped, for the most part, at the free pole of the cell. The Mankowski granules are smaller, highly refractile and are distributed in the base or throughout the cell.

The identification of the alpha and beta cells is somewhat more difficult in fresh tissue. A superficial island is located under low power by the slightly different color which the rich blood supply gives it. Under oil immersion, when the eye is dark adapted, the cells may be differentiated by the following characteristics. The beta cells which make up the majority of the cell content of the island are relatively small and well filled with tiny highly refractile granules. The nuclei are for the most part spherical and (when the chromatin may be visualized) have a considerable content of chromatin rather regularly arranged. The clear Golgi net may sometimes be visualized between the refractile granules. When the beta granules are dissolved the cytoplasm appears optically homogeneous.

The alpha cells are larger than the beta cells and tend to be elongated. The granules are larger, not very refractile and appear watery. The nuclei are oval, pale and poor in chromatin, with a prominent nucleolus. When the alpha granules are dissolved the cytoplasm becomes highly vacuolated.

The following observations were recorded.

1. Distilled water and normal salt solution rapidly remove the alpha granules while the beta, zymogen and Mankowski granules remain. On standing at room temperature there is a gradual decrement in the beta and zymogen granules, especially in distilled water.

2. Glycerine penetrates the cells slowly and the zymogen granules are lost. No changes are observed in the alpha and beta granules.

3. Dilute sodium hydroxide rapidly and dramatically removes the alpha, beta and zymogen granules. The Mankowski granules are somewhat modified.

4. With dilute acetic acid, the alpha and beta granules are preserved but with stronger acid the granules are not distinguishable and the cells show a heavy precipitate. The zymogen granules are preserved in both cases.

## TESTS OF THE SOLUTION OF THE ALPHA GRANULES

On the basis of these findings fresh pancreas was ground up with sand and extracted with distilled water, the whole centrifuged and re-centrifuged at high speeds four times and the supernatant fat skimmed off. The sediment and the suspension were examined by the stained smear method. Beta-like granules were observed in the suspension and increasingly in the sediment. The final suspension was filtered through a Berkefeld filter and collected into a sterile flask.

The total sediment, when extracted in 70% alcohol, the extract evaporated and the residue taken up in Ringer's solution and injected intravenously into a fasted rabbit, produced a fall in blood sugar equivalent to about that produced by 1 unit of insulin.

Preliminary chemical studies of the filtered extract showed: pH of about 7; a very faint Millon reaction; fine precipitation with ethyl alcohol, ether and acetic acid; no precipitation with salt solutions or dextrose.

Intravenous injection of the filtered extract produced no immediate toxic symptoms in the guinea pig, and a fall in blood sugar only within the range of experimental error.

From these observations we may conclude that:

1. Alpha granules of the guinea pig pancreas are removed by water, normal salt, 10% salt, 5% dextrose and dilute sodium hydroxide solutions and are resistant to the pure organic solvents (with the exception of ethylene glycol) and to dilute acetic acid.

2. Beta granules are removed by dilute sodium hydroxide, and 70% alcohol and are resistant to water, normal salt solution and dilute acetic acid.

3. Zymogen granules are removed by dilute sodium hydroxide, alcohol, glycerine and are resistant to water, normal salt solution and acetic acid.

4. Mankowski granules are resistant to all the aqueous solvents so far tested and to alcohol.

These properties of the secretion granules correspond to those described by Lane ('07) with the exception of the reaction of the alpha and beta granules to acetic acid. Lane believed that both types of granules were soluble in acetic acid. His negative findings may be due to a loss of staining capacity of the granules following fixation in acetic acid, or to changes in the cells produced by too great a concentration of the acid. Certainly, after fixation by Bensley's acetic-osmic-bichromate fluid both granules may be well preserved and stained by the aniline acid fuchsin-methyl green method. The properties of the Mankowski granules correspond to those described by Bensley ('14–'15).

Because the alpha granules are so labile in water and salt solutions, it becomes apparent that no methods of supravital staining or perfusion with these fluids can be adequate to demonstrate these granules. And in order to study experimental changes in the granule content of the alpha cells, every effort must be made to fix the tissue immediately.

The author wishes to express her gratitude to Prof. R. R. Bensley for his keen interest, his confirmation of observations and his technical and chemical assistance; to Dr. N. L. Hoerr for his assistance with the freezing-drying procedure; and to Dr. C. A. Woerner for blood sugar determinations.

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# FURTHER OBSERVATIONS ON THE BLOOD SUPPLY OF THE HYPOPHYSIS CEREBRI OF THE RHESUS MONKEY

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SIX FIGURES

Two previous papers dealt with the blood supply of the hypophysis of the rhesus monkey, as investigated in thick serial sections of injected pituitaries (Wislocki and King, '36; Wislocki, '36). The present contribution, which concludes these studies, employs instead, a technique of actual dissection of the hypophysis. By this method it has been possible to confirm, correct, or amplify certain of the observations made with the previous technique.

## MATERIAL AND METHOD

The blood vessels of the head of an adult rhesus monkey were injected with India ink through the heart. The calvarium was removed and the head fixed in 10% formalin. The skull was decalcified by placing it in 4% trichloracetic acid. After decalcification, the specimen was washed and carried over into 70% alcohol. With rongeurs and scissors the base of the skull was then removed, exposing the hypophysis in situ in the meninges at the base of the brain.

The final dissection of the pituitary, blocked out in situ, was carried out in 70% alcohol under a binocular dissecting microscope, utilizing iris scissors, fine jeweler's forceps and a cataract knife as instruments. The specimen under the microscope was illuminated by direct light from a Spencer spot-lamp.

## OBSERVATIONS

The results of the study of the rhesus monkey's pituitary by the technique outlined above are presented in the form of six drawings illustrating various phases of the dissection.

From examination of these figures many of the essential characteristics of the topography of the pituitary circulation can be clearly visualized.

Figure 1 shows the initial step in the dissection of the hypophysis. The bony sella and the dura (du) have been removed showing the base of the gland. In the wall of the dural cavernous sinuses (cs) the inferior hypophyseal arteries (iha) can be seen skirting the posterior border of the neural lobe, and giving off a number of small branches which, piercing the dura, penetrate the infundibular process.

In addition to the inferior hypophyseal arteries, veins can be seen in the dissection. On the surface of the gland and emanating from its substance small venules are visible. These are derived both from the anterior lobe and from the neural lobe. They pass laterally to enter the cavernous sinuses as a series of minute lateral veins (lhv).

Figure 2 represents a continuation of the dissection, obtained by carefully plucking away the infundibular process and pars intermedia, revealing a deeper set of venules (lhv) arising from the anterior lobe and the infundibulum. These also collect laterally into minute venous stems which enter the cavernous sinuses. Besides the superficial veins shown in the previous figure and the deeper veins seen here, a few similar venules were encountered, but destroyed, while removing the neural lobe. These were also directed toward the lateral border of the gland, excepting two or three which emerged independently from the posterior pole of the neural lobe. These latter venules appeared to be derived exclusively from the neural tissue and pars intermedia.

Further dissection of the hypophysis reveals the region of the stalk as shown in figure 3. This drawing illustrates the derivation of the superior hypophyseal arteries (sha) from the carotid arteries at their junction with the vessels forming

## ABBREVIATIONS

art, minute branching arteriole passing from pituitary stalk into hypothalamus
bbv, branch of basilar vein
bv, basilar veins
cs, cavernous sinus
du, edge of dura reflected from hypophysis
ev, emissary vein from hypothalamus
iha, inferior hypophyseal arteries
lhv, lateral hypophyseal veins
me, median eminence of tuber cinereum
op, optic chiasma
pv, portal venules
pvn, paraventricular nucleus
sha, superior hypophyseal arteries
v, vessel connecting pituitary stalk and hypothalamus

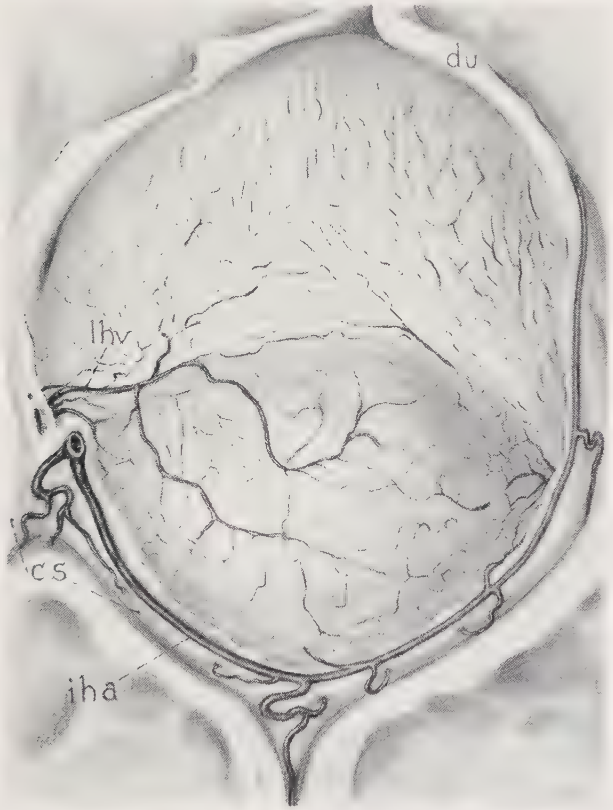

Fig. 1 Dissection of the base of the pituitary gland showing the inferior hypophyseal arteries (iha) skirting the neural lobe and giving off penetrating branches. Lateral hypophyseal venules (lhv) are seen collecting on the surface of the gland and passing laterally as minute vessels which enter the cavernous sinuses (cs).

the circle of Willis. Noteworthy is that the superior hypophyseal arteries anastomose abundantly to form an arterial plexus around the stalk, a number of arterioles finally penetrating the substance of the stalk. Clear also is the rich plexiform layer of capillaries lying in the mantle of pars tuberalis covering the stalk. This plexus is coextensive with the pars tuberalis and does not extend onto or into the surrounding tuber cinereum adjoining the attachment of the stalk to the base of the brain. Indeed the border of this plexus is conspicuously sharp. From the plexus small vessels (pv), apparently venules, arise which pass along the surface of the stalk toward the body of the hypophysis. These vessels are interpreted as being portal venules, arising from a plexus

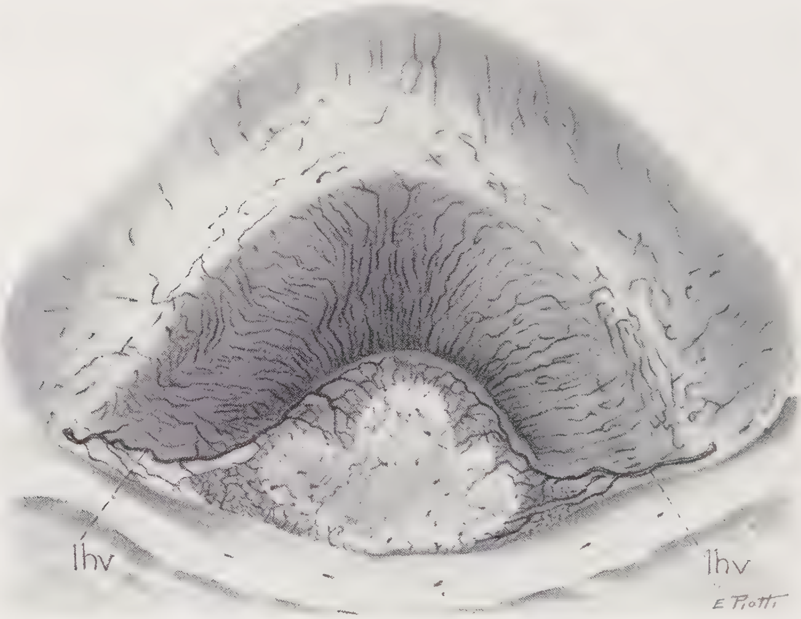

Fig. 2 Further dissection of the body of the pituitary gland, obtained by removal of the neural lobe, showing lateral hypophyseal venules (lhv) draining the interior of the hypophysis.

in the pars tuberalis and appearing to lose themselves in the sinusoidal capillaries of the anterior lobe or in the deeper capillary plexus surrounding the infundibular process.

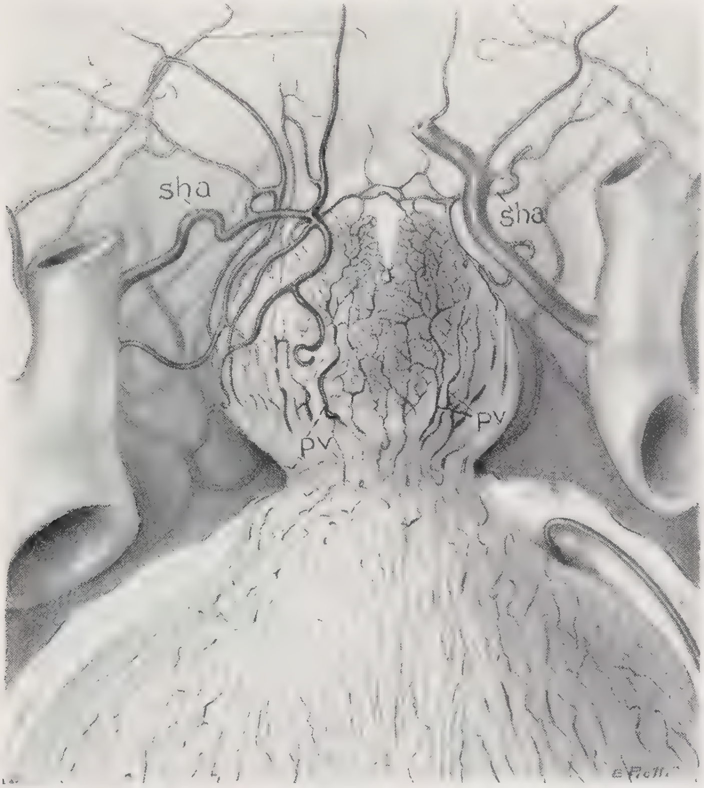

Fig. 3 Dissection of the hypophyseal stalk showing superior hypophyseal arteries (sha) derived from the carotid arteries, anastomosing and sending twigs to the hypophyseal stalk. On the surface of the stalk lodged in the mantle of pars tuberalis a rich network of vessels can be seen in which small portal venules (pv) are recognizable.

Figure 4 represents a somewhat further elaboration of the dissection showing the complete set of superior hypophyseal arteries (shv) and their numerous anastomoses. The trunks of the carotid arteries and of the circle of Willis have been in the main cut away. The terminal twigs of the arborizing hypophyseal arteries penetrate the stalk of the gland, or the neck between the stalk and the body of the gland. The body of the hypophysis has actually been cut away in the dissection, excepting a stump of pars anterior surrounding the oval infundibulum. On the cut surface of the pars anterior numerous minute vessels are seen, the larger consisting of tiny arterioles or venules, the smaller representing sinusoids. Between the anterior lobe and the oval outline of the infundibulum the cut links of a delicate plexus of capillaries are visible. The interior of the infundibulum is relatively avascular in this zone between stalk and infundibular process. At irregular intervals short delicate tufts of capillaries penetrate the infundibulum as indicated in figures 4 and 5. These tufts connect with the plexus of capillaries

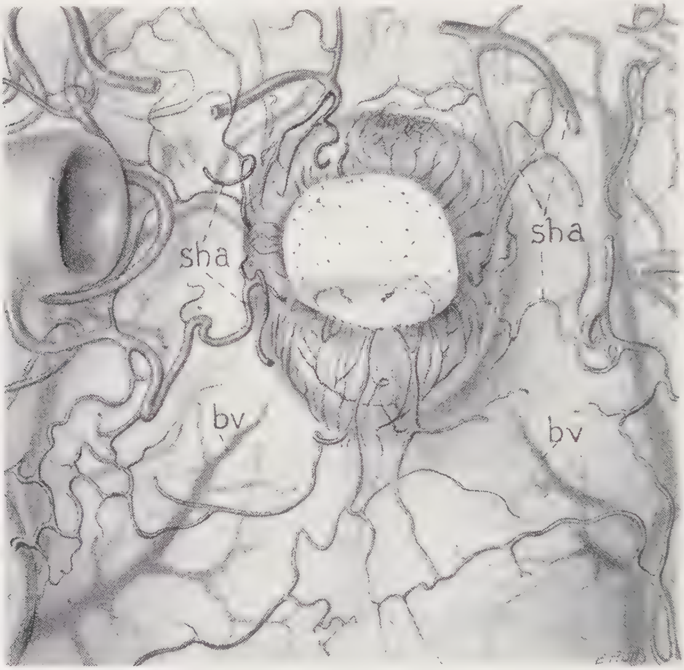

Fig. 4 The hypophyseal stalk dissected so as to show all of the superior hypophyseal arteries (sha) forming anastomoses and giving off numerous twigs to the stalk from all sides.

surrounding the infundibulum; they pierce the infundibulum anteriorly and are frequently bilaterally situated as at the two levels shown in figures 4 and 5.

Figure 5 is a companion piece to the preceding dissection. It is similar to figure 4, excepting that the arrows have been almost completely removed, revealing the distribution, course,

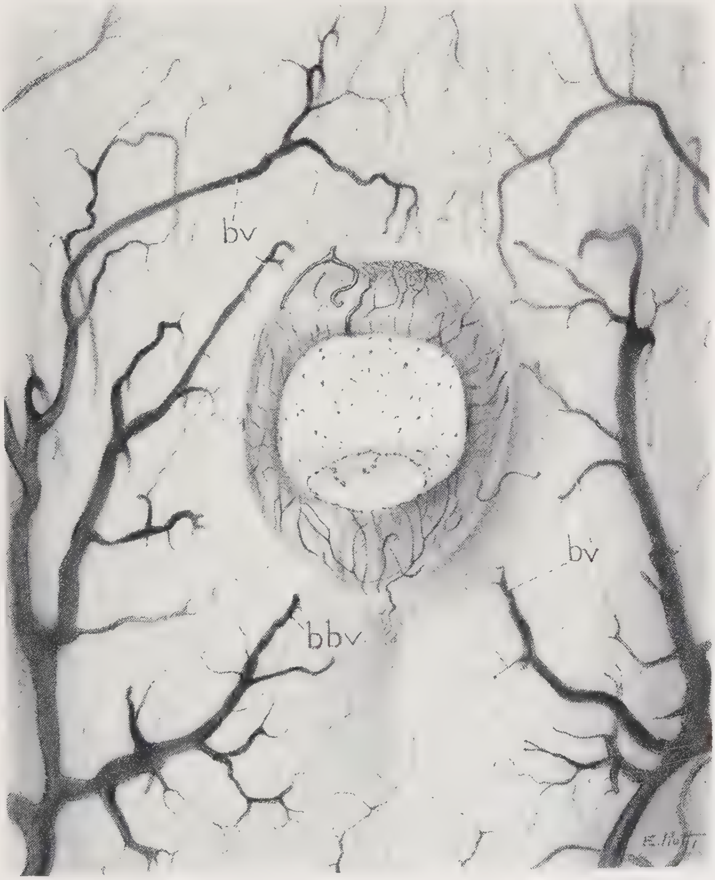

Fig. 5 A further dissection of stalk obtained by removing the superior hypophyseal arteries. Beneath the arteries is now revealed the system of basilar veins (bv) arising from venules emerging from the base of the brain, but receiving no tributary venules or capillaries from the surface of the hypophysis. A tributary venule (bbv) is shown emerging from the hypothalamus; the origin of this venule can be seen in the next figure.

and character of the basilar veins (bv) on the surface of the hypothalamus. In the preceding figure the basilar veins (fig. 4, bv) can be seen only faintly underneath the arteries at a deeper level. Noteworthy regarding these veins is that they do not arise from the hypophyseal stalk. They are formed by veins emerging from the substance of the brain and reaching the surface some distance from the stalk. These emissary venules bend at right angles on reaching the surface of the tuber cinereum and proceed, by uniting, to form the basilar veins. These circumstances account for the blunt, sudden way in which the basilar veins make their appearance, well removed from the hypophysis. Consequently there appears to be little, if any, possible venous drainage from the pituitary directly into the nearby basilar veins on the surface of the hypothalamus. The independence of the pituitary complex, as far as surface connections with these veins are concerned, is clearly seen in the figure.

The final dissection of the pituitary is shown in figure 6. The dissection is derived from the specimen portrayed in figure 5 by splitting it open in the mid-sagittal plane. Each of the resulting halves of the hypophysis reveals the interior of the third ventricle and the adjacent wall of the hypothalamus and pituitary stalk. The wall of the ventricle has been partially dissected away by removal of its most superficial substance. This brings the blood vessels in the subjacent tissue and their relationship to the blood vessels in the pituitary stalk more prominently into view. By delicate dissection the junction of the pituitary stalk and the tuber cinereum was subsequently completely dissected, in both halves, so that a survey could be made of all of the vascular connections existing between the pituitary and the hypothalamus.

Figure 6 reveals a number of interesting features regarding the vascular relationships of the pituitary and hypothalamus. First of all the characteristic plexiform tufts of sinusoidal capillaries can be seen penetrating the substance of the infundibular stalk and the median eminence (me) of the tuber

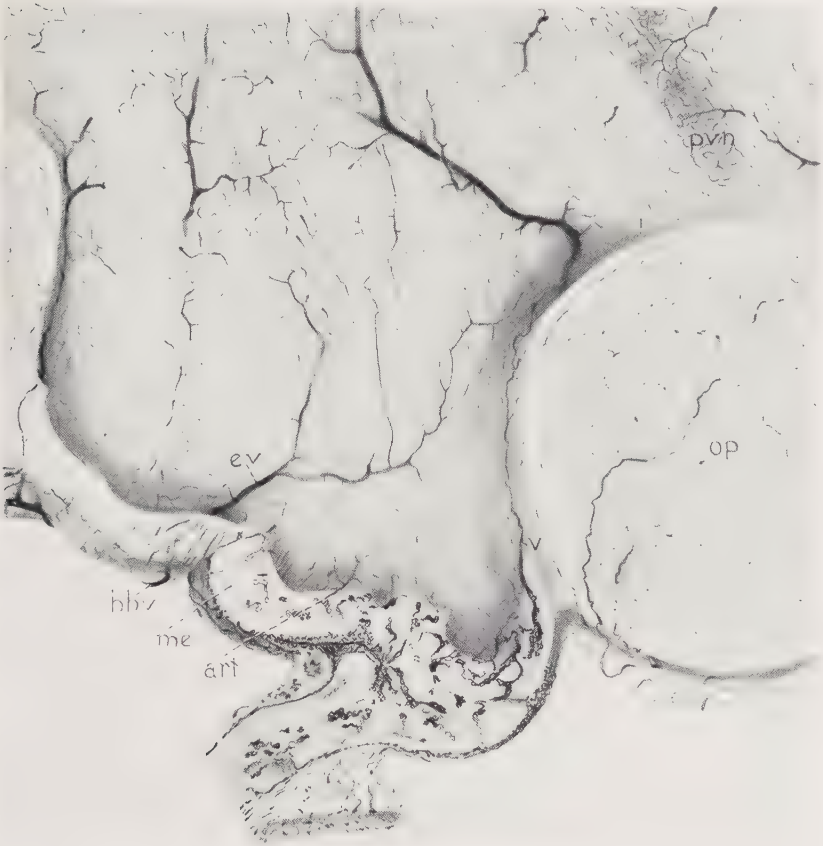

Fig. 6 The dissected hypophyseal stalk and hypothalamus split in the mid-sagittal plane, revealing the vascular plexuses within the hypophyseal stalk. The transitional vessels between the median eminence (me) of the hypophyseal stalk and the hypothalamus are shown. The largest of these (v) is a vessel of undetermined character. A minute arteriole (art) traversing the boundary of the stalk and hypothalamus is also visible. An hypothalamic vein (ev) is seen; it passes to the surface and drains into a branch (bbv) of one of the basilar veins. This branch is the same vessel seen from the surface in figure 5 (bbv). It is clear from study of the blood vessels in the transition zone between hypophyseal stalk and brain proper, shown in this figure, that there are no significant vascular links between the pituitary and the more important nuclear centers of the hypothalamus, for example, the paraventricular nucleus (pvn).

cinereum from the mantle of blood vessels and capillaries located in the pars tuberalis which encloses the hypophyseal stalk. The larger of these tufts are supplied with afferent arterioles. Secondly, it can be seen that at the border of the median eminence many of the sinusoidal tufts connect by minute vessels with characteristic slender capillaries of the brain. These anastomoses, although fairly numerous, appear in the main to be of the caliber of capillaries, only a very few of them appearing to be of the magnitude of small arterioles or venules. The second largest of all such connections between the pituitary stalk and the hypothalamus, discovered in the present specimen, is shown in figure 6 (art). It is seen to be a delicate branching vessel which emerges from the limits of the stalk and passes for a short distance into the hypothalamus before it breaks up into capillaries. It is regarded as being an arteriole for two reasons. It appears to arise from the central arteriole of a vascular tuft in the hypophyseal stalk and it breaks up into capillaries which can be traced quite definitely to the nearby hypothalamic venules (ev) shown in the figure. Only one vessel of any considerable size and extent was discoverable upon complete dissection of the entire zone of transition between the pituitary stalk and the hypothalamus proper. This vessel, which arises near the anterior border of the hypophyseal stalk, is shown in the figure (v). It emerges from a typical plexus in the median eminence of the tuber cinereum and follows a course skirting the optic chiasma. Whether it is to be regarded as arterial or venous in character remains undetermined for the nature of its origin is obscured by the complexity of the tuft from which it arises, and its ultimate destination cannot be accurately traced. It is the only extensive connection between the stalk region and the hypothalamus encountered in the course of the survey of all possible transitional vessels. Consequently it must be considered as being of exceptional occurrence.

Another noteworthy feature of the dissection shown in figure 6 is the demonstration of the complete course of an emissary vein (ev) in the wall of the third ventricle. This vein can be traced from the interior of the brain through the wall of the tuber cinereum, adjacent to the median eminence, to its connection with a branch of one of the basilar (bbv) veins on the brain surface. This same branch can be identified in the previous dissection (fig. 5, bbv). Thus we are able to trace a branch of one of the basilar veins from its entire source in the substance of the hypothalamus. Of interest regarding this typical vein is that, although it skirts the pituitary, it receives no vessels of the caliber of venules from the hypophyseal territory. Its connections with the pituitary, insofar as they exist, appear to be of capillary size only.

Regarding the possibility of sizable vascular connections between the hypophyseal stalk and the hypothalamus—especially the existence of vessels of the nature of portal veins—the present dissection of the monkey affords no convincing evidence. The anastomoses between the vessels of the two regions appear to be relatively small and quite local in character, certainly not involving any important vascular linkage between the pituitary stalk and such distantly situated hypothalamic structures as the paraventricular and supraoptic nuclei. In figure 6 part of the paraventricular nucleus (pvn) can be seen in the upper right hand corner of the field, identifiable by its pronounced capillarity. The unlikelihood of this nuclear mass receiving vessels of any size directly from the region of the pituitary stalk is apparent at a glance. And indeed this impression has been checked by careful and complete dissection of the intervening territory with the failure to locate any possible significant vascular connections whatsoever. The same negative results were obtained upon searching for possible portal venules or other direct vascular connections between the pituitary stalk and the supraoptic nuclei which lie some distance laterally to the median eminence and are not shown in the figure.

## DISCUSSION

The results of dissection of the pituitary complex by the technique adopted confirm in the main the conclusions reached in the previous studies of the pituitary of the rhesus monkey based on examination of cleared, thick, serial sections.

Little doubt remains concerning the nature and distribution of the arteries which reach the pituitary as two groups: one the inferior hypophyseal arteries; and two, the superior hypophyseal arteries. The former are distributed in the infundibular process; the latter enter the hypophyseal stalk, from which branches pass to the anterior lobe.

Further evidence is gained regarding the existence of venules passing between the hypophyseal stalk and the body of the gland. Connected at either end with capillaries, these are probably correctly interpreted as constituting a system of portal vessels.

Regarding the systemic veins, draining the hypophyseal complex, the present study reveals a series of small venules emanating from the body of the gland which enter the cavernous sinuses. These arise more especially, but nevertheless not exclusively, from the neural lobe. The stalk region appears to possess no venous connections of importance, excepting the system of portal venules connecting the stalk with the body of the gland. Direct systemic veins appear to be totally lacking. First, there is no evidence of capillaries or venules draining from the surface of the stalk into the nearby basilar veins. Secondly, direct systemic venous connections with the veins of the hypothalamus proper do not appear to exist. With the exception of one larger vessel of undetermined nature, encountered in the present specimen, and a few minute channels which are probably arterial, the vascular connections between the stalk and the hypothalamus are of capilliform size. It is apparent, moreover, that the relatively minute anastomoses between the vessels of the brain and those of the hypophyseal stalk are restricted to quite local territory, involving only the immediate border zone between stalk and brain. No significant vascular pathways exist between the pituitary and such structures, lying at a distance, as the paraventricular and supraoptic nuclei. These possess quite independent vascular supplies.

Thus it follows that in the rhesus monkey the main efferent vascular channels are those extending from the body of the gland to empty into the cavernous sinuses. Drainage by capillaries or venules connecting the stalk and the brain, if it occurs at all, must be quite subsidiary. Efferents represented by either venules or capillaries leading directly from the surface of the stalk into the adjacent basilar veins on the brain surface are completely lacking. The monkey differs in this respect from the cat in which it has recently been shown (Wislocki, '37) that a certain number of capillaries pass from the circumference of the hypophyseal stalk to the surface of the hypothalamus to become small venules tributary to the basilar veins. In spite of these efferents, the venules leading from the body of the gland into the cavernous sinuses are regarded in the cat also as being the most sizable and important pathways for venous drainage.

## SUMMARY

An account is given of the dissection, under a binocular microscope, of a pituitary gland of a rhesus monkey in which the blood vessels have been completely injected with India ink. Superior and inferior hypophyseal arteries are traced into the gland. Small venules of portal character are observed on the pituitary stalk, connecting the stalk with the body of the gland. Systemic venules are described passing from the body of the gland into the cavernous sinuses. Systemic venules running from the hypophyseal stalk to the nearby basilar veins on the surface of the tuber cinereum are lacking. Connections greater than capillary caliber between the stalk and the hypothalamus are relatively few in number and slight in extent. The largest such connections, whether they be interpreted as venules or arterioles, are too restricted in caliber, extent, or number to be regarded as playing any significant role in conveying blood from the pituitary complex to the hypothalamic centers.

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# A STUDY OF THE STRUCTURE OF THE MEDIA OF THE DISTRIBUTING ARTERIES BY THE METHOD OF MICRODISSECTION

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TWO PLATES (NINETEEN FIGURES)

## INTRODUCTION

During the microdissection of hydronephrotic rabbit kidneys it was discovered that the gentle stretching of the media of an interlobar artery resulted in its unravelling in a spiral strip several centimeters in length. This unexpected contradiction of the usual concept that the media of the distributing arteries exists in circular bundles or rings suggested a review of the record of the microscopic observations which support this notion.

Benninghoff ('30), whose classical studies provide a very careful and remarkably detailed description of arterial structure, offers only brief comment on the general arrangement of the media of the muscular arteries. This work emphasizes the circular course of the muscle and stresses the occurrence of local concentrations of elastic and white fibrous connective tissue which separate the muscle cells into bundles of varying size. It also describes the occasional occurrence of cuneiform muscle-bundles which, deviating from the circular course considered usual, are arranged in radiating fashion resembling an iris diaphragm. Bremer ('36) suggests that perhaps the muscle fibers are oblique but otherwise the literature contains only meager descriptions of the existence of sharply oblique or spiral medial muscle fibers as unusual features of isolated vessels of certain animals (Baum and Thienel, '04; Bardeleben, 1878). For these reasons an investigation of the organization of the media of the arteries by the method of microdissection seemed likely to be of interest.

## METHOD AND MATERIAL

A general survey of the normal distributing arteries of the rabbit, cat, guinea pig, monkey and infant and adult human has been made. The tissue was prepared by maceration in concentrated hydrochloric acid, washed in several changes of water, placed in distilled water in a Stender dish and examined under the dissecting microscope with the aid of needles. Fresh tissue required less maceration but yielded a preparation less advantageous than that obtained from vessels which had been promptly and thoroughly fixed in a dilute solution of formaldehyde U.S.P. (1:10). Vessels obtained from material fixed in Klotz solution were apt to be variable and often unsatisfactory in their response to maceration. An indeterminate time was required for the narrowly limited degree of softening favoring the preparation of suitable specimens and despite persistent efforts to control the procedure the most critically favorable degrees of maceration persisted in irregular appearance. The removal of air from pulmonary tissue by subjecting it to negative pressure facilitated its fixation, maceration and dissection. Fresh and formalin-fixed unmacerated vessels of each type and species were also examined in entirety and after section with a razor blade.

The tissue was examined at magnifications of × 33 and × 100 and the specimens were photographed stereographically at a magnification of × 8 while supported in the fluid in which they were prepared. The latter was never motionless and much of the detail evident upon direct examination could not be transferred to the photographic plate. Quite naturally some of the more significant observations were evident only during manipulation of the tissue and it is likewise unfortunate that they also do not permit photography.

## OBSERVATIONS

Many of the features so extensively studied by other methods present themselves with different form and emphasis when viewed in three dimensions through the dissecting microscope and certain of these observations warrant mention by virtue of their deviation from present histologic concepts.

The shortening of arteries, so striking in removal of fresh normal material, is not suggested by the usual histological examination but consequences of arterial shortening are readily evident in many of these preparations and particularly in such vessels as those of the brain which possess insignificant medial muscle and undeveloped external elastic laminae. Upon examination of these vessels the internal elastic membrane is found to present an irregular series of closely spaced, more or less transverse, outwardly convex folds which partially encircle the vessel. These transverse serial undulations of the internal elastic membrane co-exist with numerous, closely spaced longitudinal ridges of the same structure and the latter become more widely separated as they pass the summit of each undulation (fig. 1). The longitudinal folds, which are so conspicuous in the usual histological cross section of the 'contracted artery,' are found in typical form upon the immediate examination of the aorta and peripheral arteries of freshly killed animals and apparently come into existence with the fall in blood pressure. The transverse folds, also, are not infrequently encountered in vessels with thicker walls. Further, the appearance of these folds of the elastic laminae is not interfered with by treatment with acid or by heating the vessels both of which procedures are assumed by some (Evans, '26) to relax or paralyze the medial muscle.

In unmacerated fresh material the media is uncovered by spontaneous retraction of the cut edges of well-developed external elastic laminae and the latter may be everted readily by gentle manipulation exposing a considerable length of media. The union of these tunics, in many cases at least, is therefore normally less firm than might be anticipated from histologic section and after maceration the soft, highly translucent adventitia may be removed readily and without disturbance of the media. The intima in fresh material is somewhat more firmly united to the media than is the adventitia but after maceration the cylindrical remnant of intima may be withdrawn readily from the vessel. In comparison with unmacerated vessels the form of the media appears relatively unaltered by an exposure to acid which destroys the collagen and elastic tissue of the adventitia and the media retains a tensile strength indicating that the mysterious 'cement substance' uniting adjacent muscle cells is much less affected by the acid than is the adventitial connective tissue.

The normal media of most distributing arteries presents a remarkably uniform appearance with smooth surface and no obvious local variations in thickness of wall or caliber of lumen save for slight irregularities in contour due to vessel shortening. Upon dissection, the media is found to consist of sheets of obliquely arranged fusiform muscle cells and intercellular material is unidentifiable. The homogeneity and continuity of the media are not disturbed by local concentration of connective tissue and destruction of the adventitial and intimal connective tissue by acid does not result in the appearance of rings or cuneiform bundles of muscle as might be expected in accordance with the concepts of medial structure recorded by Benninghoff ('30). However, near the origin of such vessels as the iliac, mesenteric and renal arteries the smooth contour of the surface of the medial muscle is disturbed for a short distance by transverse or oblique adventitial strands which upon removal leave an irregular series of minute indentations in the surface of the muscle. In rare instances one also finds here, completely enclosed by connective tissue, coarse fusiform or crescentic, obliquely arranged muscle cell bundles about one-third of the vessel circumference in length. These groups of muscle cells, which are of most uncommon occurrence, are the only structures encountered which can reasonably be described as muscle fasciculi (fig. 2).

That the muscle fibers are not arranged transversely but rather obliquely, is indicated by the fact that the macerated media does not react to stretching by separating into perfect rings but yields a continuous, compact, spiral structure (figs. 3 to 11, 15 to 17). If the continuous coil is over-stretched rings rendered imperfect by marginal frayed ends of obliquely directed fibers result. There is little purpose in exact determination of the angle of the helix because it seems to vary in different vessels and in segments of the same vessel and because it is reduced by the vessel shortening that results on removal from the body.

Careful dissection of favorably macerated specimens discloses the successive concentric laminae of which the medial muscle is composed thus providing objective demonstration of the actuality of an arrangement that has been assumed to exist from observation of tissue sections. The successive laminae of muscle fibers are not precisely parallel. If a layer of muscle is separated and traction is applied to it, it can be unwound independently of the deeper layers in a continuous spiral progression about the longitudinal axis of the vessel. The spiral strands maintain an unbroken continuity into the branches of a vessel if these are of anywhere comparable size but such a continuity of medial fibers does not obtain in the case of small branches arising abruptly from much larger vessels.

An obscure finding is the occasional tendency of entire vessels to assume a spiral course. In figure 18 such a vessel is shown within its adventitial sheath. After removal of its adventitia attempts to unravel this vessel resulted in its fragmentation although tension applied to its duplicate resulted in the usual helical fashion (fig. 19).

That the factor of manipulation of these macerated specimens does not determine their separation into a spiral strand is evidenced by a spontaneous disruption that is often produced by a contained blood clot swelling due to the action of the acid (figs. 13 and 14). This phenomenon is not infrequently encountered but the distribution of forces is usually of such proportions as to burst the vessel along one side in which event there is opportunity to observe the constant angles of the serrations presented by individual cells at the margin of the separated muscle. This seems to indicate that adjacent muscle cells are constantly arranged with regard to their central and terminal portions.

An interesting deviation is provided by the pulmonary artery of the guinea pig. The first portion of this vessel is of relatively large caliber and possesses an exceeding thin wall. This condition obtains through the first three or four branchings within a lobe. Upon dissection the delicate media also is found to exhibit a spiral progression and to be of uniform character. After the third or fourth branching, however, there occurs a marked reduction in caliber and a considerable and absolute increase in thickness of the media which is now arranged in cylinders or segmental rings of inconstant length. Occasionally a tenuous thread of muscle is demonstrable connecting the segments (fig. 12).

## DISCUSSION

In this study of the distributing arteries of a number of animal species the media was found without exception to lack a fascicular arrangement and to be composed of compact, concentric laminae of obliquely directed, fusiform muscle cells united so closely as to support suggestions of the syncytial character of the muscle. The cells are organized into a continuous spiral structure.

The interruption of the medial muscle in the guinea pig lung is evident in usual microscopic sections and has been described (Jordan, '30) also in the ox, sheep and pig in which the segmented condition of the media is said to be modified by being spirally disposed. The functional possibilities of such an interrupted medial muscle as is found in the guinea pig lung must obviously be different from that of muscle which exists as a syncytium and thus attention is directed to the probable importance of the lack of local concentration of connective tissue which has so casually been believed to exist in the media.

Viewed directly through the dissecting microscope, the transverse and longitudinal folds of the internal elastic membrane seem a result of its intrinsic elastic qualities and not to be an especial consequence of post-mortem muscular contraction as is commonly assumed. The assumption of MacWilliam ('02, '08), Galloway ('36) and others to the effect that the internal elastic lamina is passive and that the number and depth of its folds are acceptable evidence of the degree of a hypothetical post-mortem contraction occurring in the musculature of the media is not supported by the present evidence. It would seem that the folds of the internal elastic lamina of the muscular artery are reasonably comparable to the similar folds occurring in the elastic laminae of the aorta which is relatively lacking in muscular elements.

Consideration of the possible functional significance of the organization of the arterial muscle into a continuous spiral structure obviously involves one of the most intricate problems in physiology being primarily concerned with the question as to whether or not the development of the heart has rendered unnecessary the original contractile function of the arteries and if, as is commonly assumed, their function has become limited to adapting the caliber of the vessel to the volume of the blood and to its distribution in accordance with the requirements of the various organs.

The possibility of distributing arteries contributing to the forward movement of the blood by their intrinsic motor activity has been the subject of considerable speculation, confined largely to the concept of an active arterial systole or peristaltic arterial contraction which, to avoid obvious conflict with the evidence of arterial tracings, has usually been postulated as coordinate with the impulse of ventricular systole.

On a physical basis Hess ('16, '17, '19) believes that lack of arterial valves is of decisive significance in this problem and, since effective peristaltic contraction necessarily needs to be of such amplitude as to nearly close the vessel lumen and of a velocity equal to that of the blood flow points out that such waves have not been observed in the larger arteries of vertebrates. He found no support of the concept in analysis of arterial tracings. He observed, as others had done, rhythmic peristaltic pulsations in veins of the wings of bats but he found no comparable activity in the ear vessels of rabbits although such had been reported by other workers. Further, the curves obtained from pulsating bat wing veins differed distinctly from the usual arterial tracings.

Fleisch ('20) measured arterial cross section area and blood pressure curves and found a perceptible lag in the former which caused him to interpret the cross section change in the vessel as purely passive.

On the other hand certain investigators have suggested that distributing arteries do not react as passive tubes. Hürthle ('12, '15) determined velocity and pressure relations in models and discovered discrepancies when he compared his calculations with volume of blood flow and pressure variations during systole and diastole. He found this discrepancy lacking in vessels after death or 'paralysis' and that it was accentuated by epinephrine and pituitrin. This led him to consider the possibility that the discrepancy might be due to a physiological cause such as muscular contraction, but finally concluded that the possibility of active participation of the arterial muscle in furthering progress of the blood remains a possible but still unproven hypothesis.

Hasebroeck ('14) and Mareš ('16) support the possibility of arterial systole but offer no conclusive evidence favoring their ideas. Nevertheless, Mareš presents some pertinent general comment in pointing out that arteries are not inert tubes and that speculation as to their function should take into consideration their known structure. He further mentions the uncertainty of the opposite views, criticizes Hess' generalizations from negative findings and calls attention to the paradox of the ready acceptance of the equally difficult mechanical riddle of vasodilatation in contrast to the ready dismissal of the possibility of arterial systole.

MacCallum ('36) has long maintained that the arterial muscle aids the heart by a sort of peristaltic contraction and invokes this mechanism as a partial explanation of the passage of blood into the venous circulation after the cessation of the heart beat.

The validity of the concept of arterial systole is most seriously disturbed by the obvious conflict between the recognized sluggish character of smooth muscle contraction and the high frequency required to accompany pulse variations and Wiggers ('34) points out that the variation in velocity of pressure transmission would require assumption of changes in rate of conduction unknown in physiology. In this regard it is to be observed however, that Evans ('26) emphasizes the indefinite nature of our knowledge of smooth muscle, the danger of drawing general conclusions from the behavior of isolated examples, the probability that there is more than one kind of smooth muscle and the variability of the period of latency, contraction and relaxation in muscle of different types and with different stimuli.

There has been considerable discussion of the rhythmic contractions observed in certain blood vessels apparently independent of the central nervous system and Howell (1896) at one time suggested that they may be purely muscular in origin. It would also seem possible that the slower, rhythmic pulsations known as Traube-Hering waves may be due to independent muscular activity. Wigger's ('34) states, "Crucial proof that these rhythmical variations are in fact due to variable vasomotor activity remains to be adduced."

It may be that the spiral arrangement of the muscle is of significance in relation to the mechanism of conduction essential to any coordination of contractile elements. The latter is especially obscure in the instance of the arterial media. The nervous mechanism is poorly defined in both its structural and functional aspects and there is also the possibility of direct conduction through the muscle cells. From the time of Volkmann (1850) it has been suggested that distension of the vessel by the pulse wave might serve as a dominant, coordinating stimulus to the contraction of the muscle cell.

Several workers believe that intestinal muscle shows rhythmic contraction without nerve participation. Bozler ('37) in the instance of the uterus, ureter and intestinal muscle, offers evidence that there is a slow propagation of stimulus directly through the muscle cells and that this stimulus passes much more readily in the longitudinal axis of the cell. If confirmed, the implications of this latter work are of especial importance with respect to the functional possibilities of the spiral medial muscle.

The spiral organization of the medial muscle has been found in the smallest vessels possible to dissect and it is reasonable to suppose that this arrangement may be continued into the smallest arteriole. Muscle has, in fact, been pictured as spirally encircling such vessels by Zimmerman ('23) and by Maximow after Retzius ('30). Zimmerman describes this relationship in a 25 μ thick branch of a coronary artery of an adult human and Retzius records it as occurring in a tiny artery from the vascular membrane of a rabbit's eye. In smaller vessels rhythmic contractions have been observed to participate in the movement of the blood although the relationship of blood movement to the spiral arrangement of the muscle cells has not been recognized. It is reasonable, therefore, to suggest that a contraction wave spreading from one end of such a spirally arranged muscle cell could provide a mechanism leading to the onward movement of the blood and further to draw a parallel between such a simplified example and the spirally arranged muscle of the larger vessels for it is well known that excised strips of arteries undergo slow, spontaneous, rhythmic contraction under favorable conditions.

Such a suggestion seems permissible in view of the nebulous state of our knowledge of haemodynamics for as Fleisch states, "In jedes Gesetz über Blutkreislauf würde sich der eine unbekannte Faktor "aktive Förderung des Blutstromes durch die Gefässe" einschleichen und die Auswertung des Gesetzes illusorich machen."

## CONCLUSIONS

1. The internal elastic lamina of most distributing arteries presents not only longitudinal but also transverse folds which seem due to the intrinsic elastic character of the membrane and not to post-mortem muscular contraction as is commonly assumed.
2. The media of the distributing arteries lacks local concentration of connective tissue and is composed of concentric laminae of obliquely directed fusiform muscle cells united so closely as to suggest a syncytial character and organized into a continuous spiral structure.
3. On spontaneous disruption of the media due to action of acid on contained blood clots the media yields a spiral structure.

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# PLATES

## PLATE 1

### EXPLANATION OF FIGURES

1 Camera lucida drawing of macerated branches of adult human cerebral arteries from which the scanty adventitia has been stripped. The insignificant medial muscle has been displaced by transverse and longitudinal folding of the internal elastic lamina. Similar foldings of these and other vessels have been noted both immediately after death and after maceration. × 16.

2 The only muscle fasciculi encountered in this study were these unimpressive structures obtained from the outer surface of the media of the iliac artery of a monkey. A few similar bundles were also found in the initial portion of the mesenteric, celiac and renal arteries. × 16.

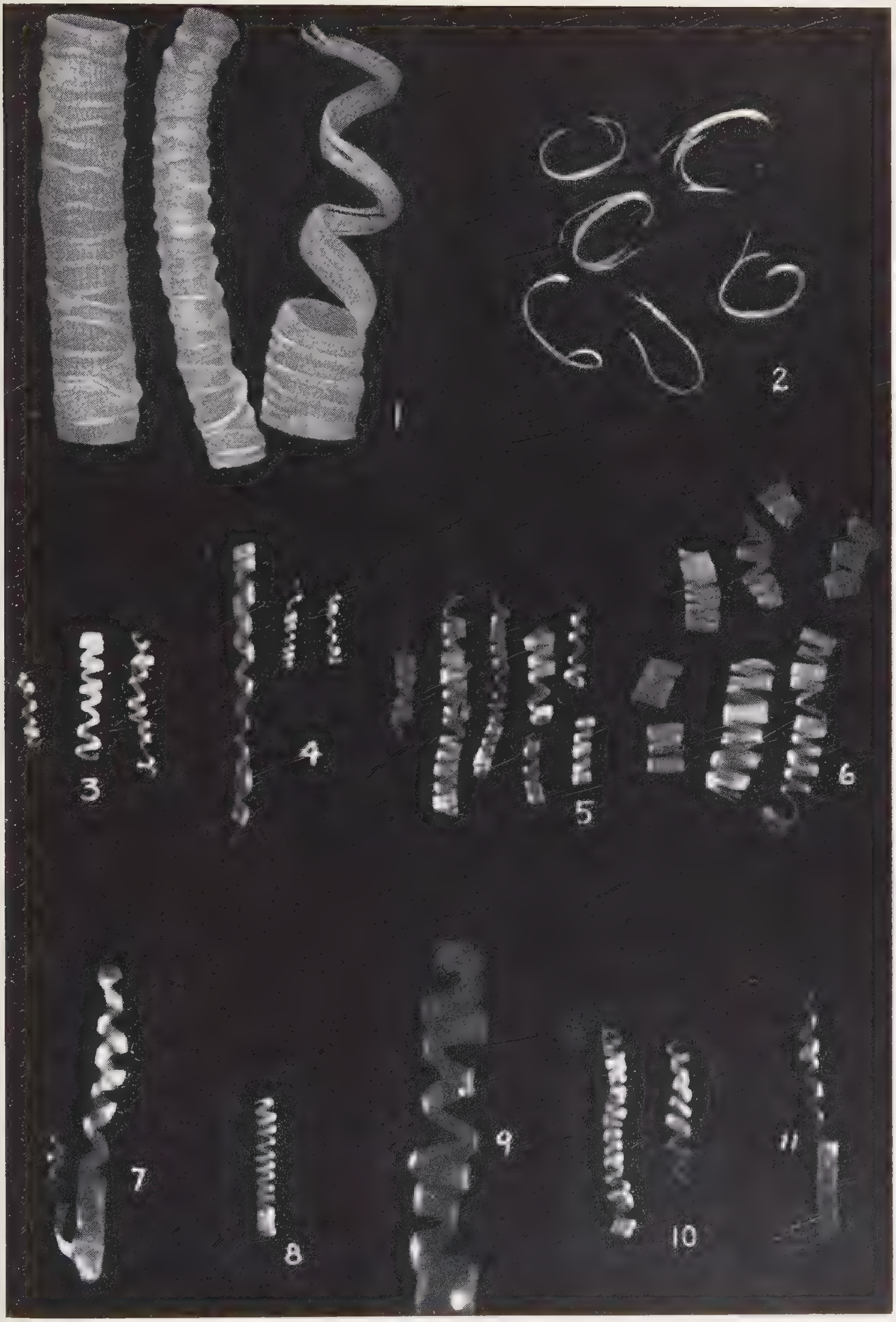
3, 4 and 5 Spiral strands obtained by stretching the macerated media of the hepatic, mesenteric and splenic arteries of the guinea pig. × 8.

6 Spiral strand obtained by stretching the macerated media of a branch of a cerebral artery of a human adult. × 8.

7 Same, coronary arterial branch, human adult. × 8.

8 Same, celiac artery, newborn human. × 8.

9, 10 and 11 Same, carotid, splenic and renal arteries, cat. × 8.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## PLATE 2

### EXPLANATION OF FIGURES

12 Drawing made from stereoscopic photographs of a branch of the pulmonary artery of the guinea pig illustrating the segmental distribution of the medial muscle, × 16. (Contrast with the drawing (Jordan, '30) reconstructed from tissue sections.)

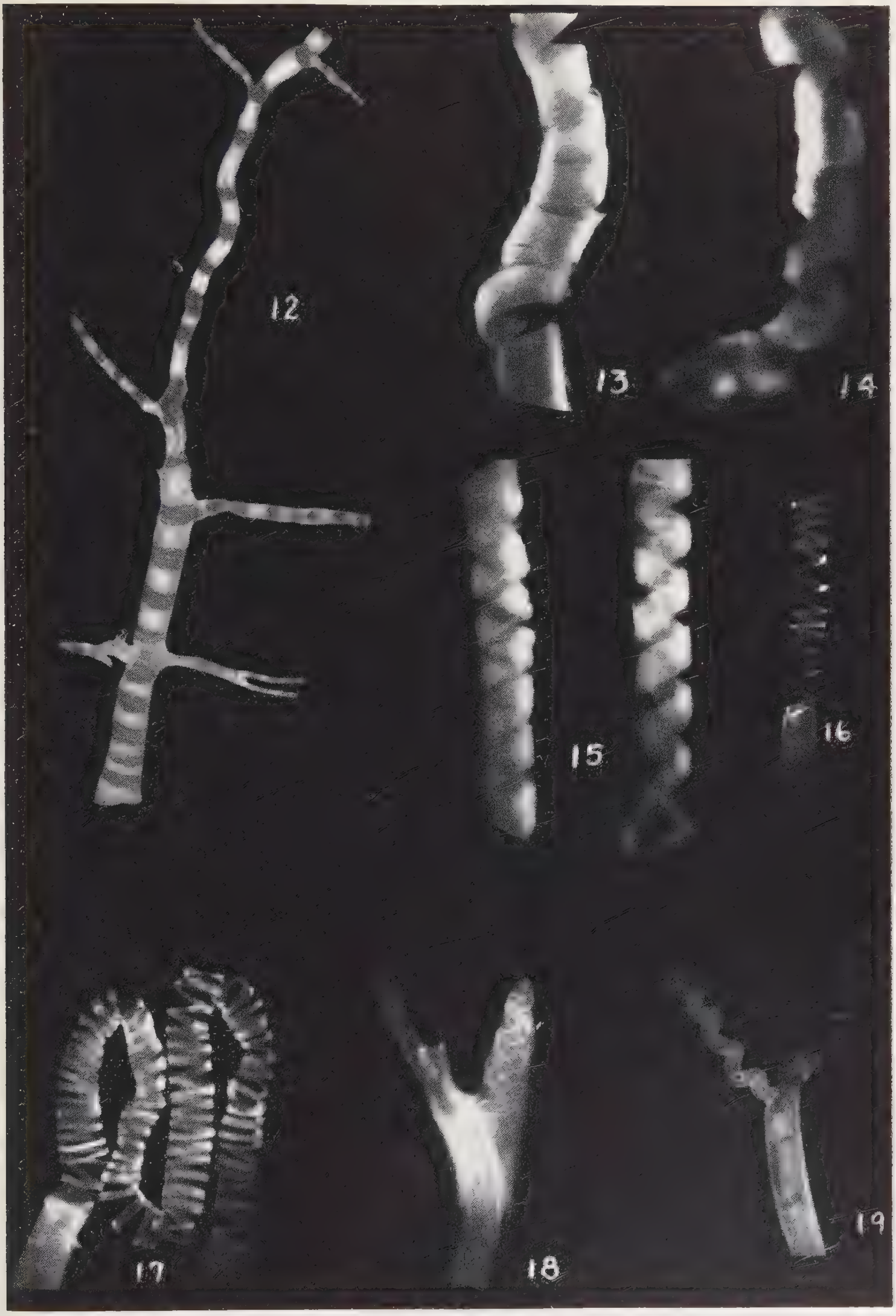
13 and 14 Spontaneous disruption of the femoral arteries of the monkey and rabbit produced by the swelling of contained blood clot due to the action of the acid. × 8.

15 Spiral strands obtained by stretching the macerated media of the femoral artery of the rabbit. × 8.

16 Same, splenic artery, monkey. × 8.

17 Same, internal iliac artery, monkey. × 8.

18 and 19 Illustrates the curious tendency of some vessels to assume a spiral course. The vessel is shown within its adventitial sheath and again after removal of the adventitia and after application of tension to its duplicate branch. × 8.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# QUANTITATIVE MEASUREMENTS OF THE FAT-LADEN AND FAT-FREE SEGMENTS OF THE PROXIMAL TUBULE IN THE NEPHRON OF THE CAT AND DOG[1]

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FIVE FIGURES

It has been adequately demonstrated that the proximal tubule[3] of the cat and dog nephron characteristically contains fat under normal conditions, and that this fat is regularly distributed in a particular manner in the two species (Peter, '09; Zimmermann, '11; Nakamura, '35). In the cat the entire pars convoluta and the upper portion of the pars recta are normally fat-laden, while the terminal portion of the pars recta is fat-free. In the dog the reverse is true, the fat being confined exclusively to the terminal portion of the pars recta. In tubules teased from acid maceration material the transition between the fat-laden and fat-free portions is characteristically abrupt (Peter, Nakamura). Zimmermann added greatly to our knowledge by demonstrating that the abrupt transition with respect to fat content is likewise abrupt with respect to cell type. In both species the luminal cell boundary pattern in the terminal portion of the pars recta—fat-free in the cat, fat-laden in the dog—is rectilinear. In the remainder of the proximal tubule—fat-laden in the cat, fat-free in the dog—the luminal cell boundary pattern is very complicated,

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[1] A brief preliminary account of this study has been published by Foote ('36).

[2] Fellow of the Henry Strong Denison Medical Foundation.

[3] 'Proximal tubule' will be used in this paper to designate the entire portion of the nephron provided with brush border, extending from the glomerulus to the transition to the thin segment.

showing in marked degree the interdigitations usually considered characteristic of the mammalian proximal tubule. Despite the reversal in fat distribution in the two species, these findings seem amply to justify the conclusion that we are dealing here with two distinct segments of the proximal tubule. Whether the bulk of the proximal tubule (exclusive of the terminal portion of the pars recta) can be further subdivided on cytological grounds must await further work. As is well known, Suzuki ('12) has subdivided the mammalian proximal tubule into three, or in the rabbit four, segments, but no critical cytological proof of his thesis has yet been adduced. In a re-investigation of the problem—the details of which will be published at a later date—we have up to the present time been forced to conclude that, in the cat and dog, there are only two cytologically distinct segments in the proximal tubule, namely, the two established by Zimmermann. Since, in both cat and dog, the abrupt shift in fat distribution demarcates so accurately the cytological transition between the upper and lower segments, we have used isolated proximal tubules stained for fat as a means of obtaining quantitative measurements of the relative lengths of the two segments.

## MATERIAL AND METHODS

The cats and dogs used for this study were apparently healthy adults, taken immediately before use from the animal farm, where they had been maintained for varying lengths of time on the routine diet. Cats: no. 4, male, 3.6 kg.; no. 5, male, 3.8 kg.; no. 6, female, 2.5 kg.; no. 7, female, pregnant (near term), 3.7 kg. Dogs: no. 1, female, 6.2 kg.; no. 2, male, 8.2 kg.; no. 4, female, 10.0 kg.; no. 5, female, 11.6 kg.; no. 6, male, 9.7 kg. The cats were killed instantaneously by mechanical means, and the dogs were killed by illuminating gas. The kidneys were removed at once and cut into thin (2 to 3 mm.) strips, which were placed in 10% formalin and kept there until used. Urine aspirated from the intact bladder of all animals gave a negative test for protein.

Small blocks of these formalin-fixed kidneys, extending from the capsule well into the medulla, were macerated in concentrated hydrochloric acid (specific gravity 1.18 to 1.19);

the length of time required varied considerably with different blocks, and estimation of the proper degree of maceration had to be learned by experience. The blocks were washed free of acid with many changes of distilled water, and then stained with either Sudan III (saturated solution in 70% alcohol with 1% KOH) or Scharlach R (saturated solution in 70% alcohol with 2% NaOH) for periods of from 3 to 5 hours. The blocks were then retransferred to distilled water and allowed to 'puff' until suitable for teasing. From the stained blocks complete proximal tubules, with the attached glomerulus and with a portion of the thin segment of Henle's loop, were teased and mounted under the dissecting microscope on cover slips smeared with a thin film of glycerine-albumen mixture. These cover slips were then mounted on slides in glycerine diluted with water (for greater ease in handling). Finally, within a short time after the preparations were made, since they do not last, the outlines of the entire proximal tubules were drawn with the aid of the camera lucida, at a magnification varying between × 170 and × 180 (accurately determined for each drawing). The point of abrupt shift in fat distribution was noted on the drawings, and from the latter the desired measurements were made with a Keuffel and Esser map measurer. The figures given in the tables were calculated from the measurements made on the drawings. The point of transition from the proximal tubule to the thin segment is sharply defined, and can be determined without difficulty.

## OBSERVATIONS

In the following discussion the major portion—entire pars convoluta and upper portion of pars recta—of the proximal tubule will be referred to as the 'first segment,' and the terminal portion of the pars recta of the proximal tubule will be referred to as the 'second segment.' As explained in the introduction, the first segment is normally fat-laden in the cat, fat-free in the dog; and the second segment is normally fat-free in the cat, fat-laden in the dog.

### *Cat*

By the methods described above, sixty complete proximal tubules—fifteen from each of four cats—were isolated, mounted, drawn and measured, the lengths of the first and second segments being determined by the fat distribution. The results are summarized in table 1. The average percentage length of the second segment in each of the four animals is 17.7, 15.5, 20.7 and 23.9% respectively. All of the tubules from cats nos. 4 and 5 were isolated from the central portion of the kidney, i.e., the region directly opposite the hilus and approximately equidistant from the poles. In the other two animals groups of tubules were isolated, for comparison, from the central and polar regions of the kidney on the one hand (cat no. 6), and from the central and lateral regions of the kidney on the other hand (cat no. 7). Since the measurements of the tubules in the different groups showed no significant differences dependent upon the location of the nephrons in the kidney substance, all of the tubules isolated from cats nos. 6 and 7 have been grouped together in the table.

TABLE 1

*Total length, and length of first and second segments, of proximal tubule of cat nephron; fifteen proximal tubules from each cat; all measurements in millimeters*

| CAT NO. | TOTAL LENGTH PROXIMAL TUBULE | LENGTH OF 1ST SEGMENT | LENGTH OF 2ND SEGMENT | 2ND SEGMENT—PER CENT OF TOTAL LENGTH |
|---|---|---|---|---|
| 4 | 8.83–13.22 | 6.61–11.89 | 1.33–2.90 | 13.3–25.0 |
| 5 | 11.42–16.10 | 9.62–13.70 | 1.27–3.83 | 9.8–28.7 |
| 6 | 7.07–11.10 | 5.37– 9.12 | 1.39–2.54 | 14.8–27.5 |
| 7 | 6.70– 9.92 | 4.71– 8.25 | 1.58–2.76 | 16.9–31.8 |
| Range | 6.70–16.10 | 4.71–13.70 | 1.27–3.83 | 9.8–31.8 |
| Mean for 60 tubules | 10.81 mm. | 8.79 mm. | 2.02 mm. | 19.5% |

The distribution of fat in the proximal tubule of the cat nephron was found to be relatively constant. The typical findings may be summarized as follows. At the glomerular end the fat begins abruptly with no apparent neck segment. The fat droplets here are of both small and medium size and are quite numerous. For a short distance distally along the tubule they gradually increase in size, their numbers remaining essentially the same. After the fat deposit has become heavy it remains so until near the distal end of the first segment, where the fat gradually diminishes. At the end of the first segment the fat stops abruptly and completely; the second segment is wholly fat-free.[4]

Although most of the intersegmental transitions observed were abrupt (figs. 1 to 3), occasional variations were encountered in each of the four cats. In some cases the cell type shifted at different levels on the two sides of the tubule, several fat-laden cells appearing opposite fat-free cells. In a few instances 'double' transitions (fig. 4) were noted: the cell type changed abruptly—from that of the first to that of the second segment—around the whole circumference of the tubule, then a short distance farther on reverted to that of the first segment, finally to change again to that of the second segment, this type then continuing on to the transition to the thin segment. It should be emphasized, however, that these variations are rare.

In most instances, the proximal tubule exhibited a distinct, though slight, increase in diameter at the point of transition from the first to the second segment (fig. 1). In one animal (cat no. 5) this increase in diameter was very marked (figs. 2 to 4). This animal likewise showed diverticula of the proximal tubule as a constant feature. These diverticula regularly occurred in the distal third of the first segment, and usually numbered about fifteen to each tubule. They ranged in size from slight irregularities in the contour of the tubule to structures four or five tubule diameters in length (fig. 5). Occasionally in the animal diverticula were also noted in the second segment (fig. 3). When they appeared here they were invariably close to the point of transition. The diverticula always exhibited the type of epithelium (with respect to fat content) characteristic of the segment of the proximal tubule from

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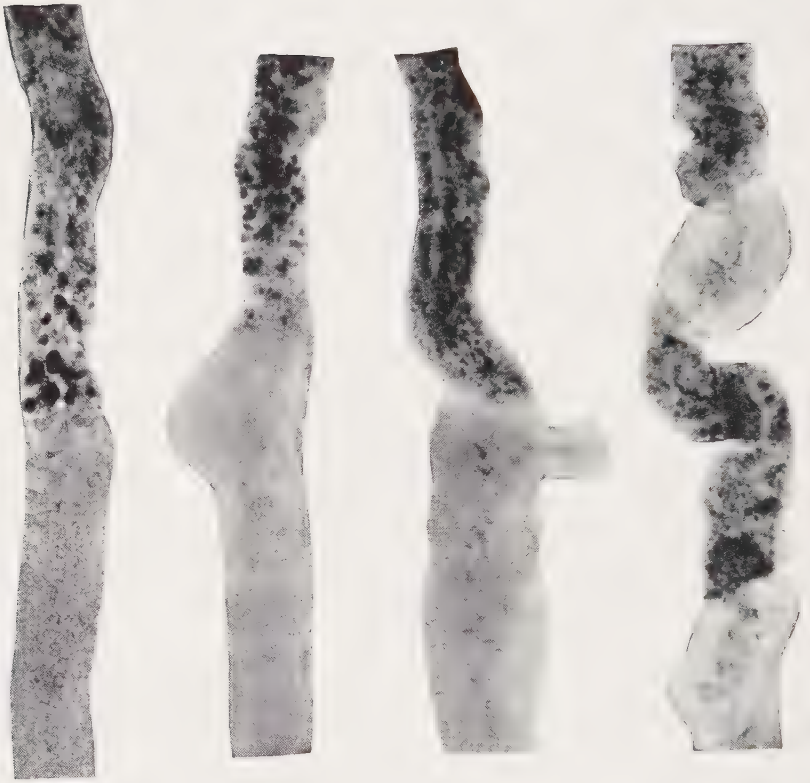
[4] In a cat (not one of the present series) which was killed with chloroform and ether instead of by mechanical means, some fat droplets were found in the cells at the distal end of the second segment in about 90% of the proximal tubules examined. These droplets became fairly numerous toward, but stopped abruptly at, the transition to the thin segment. In no instance in the four cats reported in this study was any fat found in the second segment.



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Figures 1 to 4 (from left to right). Transitions from fat-laden (first) to fat-free (second) segment of proximal tubule of cat nephron; teased preparations from maceration material.

Fig. 1 Characteristic transition, showing abrupt shift in fat distribution and somewhat larger diameter of second segment. × 350.

Fig. 2 Transition showing marked dilatation of second segment at point of transition. × 200.

Fig. 3 Transition showing small diverticulum of first portion of second segment. × 200.

Fig. 4 'Double' transition (fat-laden, fat-free, fat-laden, fat-free); see text. × 200.

Figure 1, from cat no. 1, stained with Sudan III; figures 2, 3 and 4, from cat no. 5, stained with Scharlach R.

which they arose. In no other animal studied were these diverticula encountered.

In the course of dissecting the proximal tubules under the observation microscope, it was noted that the transition between the first and second segments typically occurs within the medullary ray. There is no definite level at which it occurs in all tubules. In general it may be said that the tubules originating from glomeruli near the periphery of the cortex have their transition nearer the upper end of the ray than those originating from lower-lying glomeruli. It was further noted that the 'pars recta' of some of the proximal tubules does not run a straight course, but is of a spiral or corkscrew shape, as originally emphasized by Schachowa (1876). Proximal tubules originating from glomeruli lying near the medulla almost invariably show this arrangement of their 'pars recta.' All variations from slight bends to a marked corkscrew shape are encountered, the most marked distortion occurring in those tubules originating from the lowest-lying glomeruli.

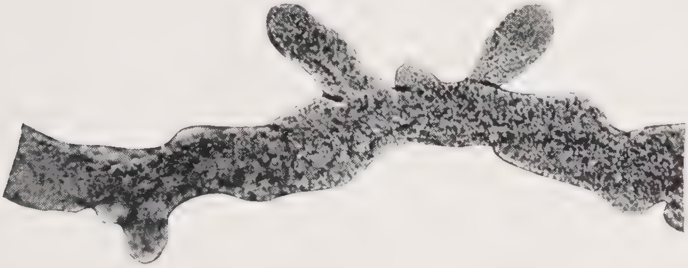

Fig. 5 Diverticula of the distal third of the first (fat-laden) segment of the proximal tubule of the cat nephron. These diverticula were among the largest observed. From cat no. 5; teased preparation from maceration material, stained with Scharlach R. × 200.

### *Dog*

For the dog, as for the cat, sixty complete proximal tubules —fifteen from each of four dogs—were isolated, mounted, drawn and measured. The results are summarized in table 2. The average percentage length of the second segment in each of the four animals is 13.9, 10.0, 9.1 and 10.3% respectively.

Most of the tubules were isolated from the central portion of the kidney; the remainder were isolated from other regions. All of the tubules from a given animal have been grouped together in the table, since in the dog—as in the cat—the location of the nephron in the kidney substance is apparently without particular significance for this aspect of the problem.

As discussed in the introduction, the first segment of the dog's proximal tubule is typically fat-free, the second segment typically fat-laden, and the transition between the two segments is abrupt. In dogs 1 and 4 these findings were the rule, the second segment showing an accumulation of large fat droplets just below the point of transition. In some instances this initial heavy deposit shaded off gradually as the transition to the thin segment was approached, the terminal portion of the second segment being at times fat-free. In other instances, the initial heavy deposit was not continuous, being interrupted at intervals by groups of cells containing little or no fat. The findings in dog 6 were essentially the same as in dogs 1 and 4, with the additional observation that some of the tubules showed a heavy accumulation of very fine—rather than large—fat droplets at the beginning of the second segment. The tubules of dog 5 were atypical in that the first segment—over approximately one-quarter of its length, beginning at the glomerulus—showed very fine fat

TABLE 2

*Total length, and length of first and second segments, of proximal tubule of dog nephron; fifteen proximal tubules from each dog; all measurements in millimeters*

| DOG NO. | TOTAL LENGTH PROXIMAL TUBULE | LENGTH OF 1ST SEGMENT | LENGTH OF 2ND SEGMENT | 2ND SEGMENT—PER CENT OF TOTAL LENGTH |
|---|---|---|---|---|
| 1 | 16.68–21.60 | 13.82–18.41 | 1.86–3.30 | 9.30–17.10 |
| 4 | 13.02–16.61 | 11.68–15.24 | 1.01–1.67 | 6.84–11.66 |
| 5 | 22.00–36.80 | 19.21–34.50 | 1.36–3.14 | 4.47–13.90 |
| 6 | 26.70–33.00 | 23.90–28.95 | 2.30–4.30 | 6.85–13.90 |
| Range | 13.02–36.80 | 11.68–34.50 | 1.01–4.30 | 4.47–13.90 |
| Mean for 60 tubules | 22.50 mm. | 20.14 mm. | 2.36 mm. | 10.8% |

droplets, appearing under the dissecting microscope as a diffuse staining. These droplets gradually diminished in number as one proceeded distally, and the remaining three-quarters of the first segment were wholly fat-free. The second segment showed a heavy deposit of fat droplets throughout. These droplets were predominantly small, but interspersed among them were larger ones, occurring either singly or in small groups (up to ten in number). In all four of the animals the two segments under consideration stood out quite clearly and could be measured without difficulty.[5]

In the dog, as in the cat, the 'pars recta' of the proximal tubule was not infrequently of a spiral or corkscrew shape. Where marked distortion was found, it could be established that these tubules regularly originated from glomeruli lying deep in the cortex.

## DISCUSSION

As is well known, the effects of the acid maceration technic upon tissues—particularly upon a tissue such as the kidney—are highly variable with respect to the degree of swelling obtained. This being true, we should like to emphasize that the absolute lengths of the proximal tubules recorded in tables 1 and 2 are not to be considered in any sense representative of the actual lengths of the tubules in the living, or even in the fixed, organ. On the other hand, we have no reason to suspect that the two segments of the proximal tubule are disproportionately altered in length by the maceration technic to any significant extent. Under the circumstances, we believe that the percentage lengths of the two segments as obtained by this method are valid.

If we accept this conclusion, there is an additional fact to be derived from the data in tables 1 and 2 which is worthy of

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[5] In another dog (no. 2) no fat was demonstrable anywhere in the proximal tubule. That such a fat-free condition of the pars recta can be attained, e.g., on a deficient diet, was emphasized long ago by Schachowa (1876), who deliberately produced the condition in order to study, under more ideal conditions, the shapes of the cells in this portion of the nephron.

mention. The average percentage length of the second segment is almost twice as great in the cat (19.5%) as it is in the dog (10.8%); on the other hand, the average total length of the proximal tubule is roughly twice as great in the dog (22.5 mm.) as in the cat (10.8 mm.). Thus, despite the disproportion in average percentage length of the second segment in the two species, the average length (in millimeters) of this second segment is nearly the same for both animals (2.02 mm., cat; 2.36 mm., dog). What significance this observation may have must remain purely speculative for the present, but the fact is clear.

No attempt will be made here to review the extensive literature upon the occurrence of fat in the kidneys of the cat and dog. The more important references can be found in von Möllendorff ('30) and Nakamura ('35). In his recent paper Modell ('33) concludes that "large amounts of lipoid droplets are demonstrable in the convoluted portions of the renal tubule of normal adult cats, and are absent in the other portions of the tubule." This statement is obviously incomplete as concerns the proximal tubule, since the upper portion of the pars recta is likewise fat-laden under normal conditions.

## SUMMARY

The proximal tubule of the cat and dog nephron characteristically contains fat under normal conditions, and this fat is regularly distributed in a particular manner in the two species. In the cat the entire pars convoluta and the upper portion of the pars recta are normally fat-laden, while the terminal portion of the pars recta is fat-free. In the dog the reverse is true, the fat being confined solely to the terminal portion of the pars recta. The transition between the fat-laden and fat-free portions is abrupt, not only with respect to fat content, but also with respect to cell type (Zimmermann). Despite the reversal in fat distribution in the two species, the findings seem to justify the conclusion that we are dealing with two cytologically distinct segments of the proximal tubule.

In the present study the attempt has been made to obtain a quantitative estimate of the relative lengths of these two segments in the adult. From each species sixty complete proximal tubules—fifteen from each of four animals—have been isolated from maceration material and measured. The tubules were stained for fat, and by this means the point of transition between the two segments could be readily identified. In percentage of the total length of the proximal tubule, the average length of the second segment is 19.5% in the cat, 10.8% in the dog.

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# THE EPITHELIAL COMPONENTS OF THE PITUITARY GLAND OF THE OPOSSUM

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TWO TEXT FIGURES AND ONE PLATE (SIX FIGURES)

In a previous paper (Dawson, '37) attention was directed to the presence of a relatively well-defined zone in the median anteroventral region of the pars distalis in the pituitary gland of the rabbit and cat. This region was characterized by the restricted distribution of acidophiles and predominance of basophiles and chromophobes and was called the zona tuberalis on account of its morphological relationships to the pars tuberalis.

It was not possible, in either the rabbit or cat, to correlate the presence of this area with the developmental history of the gland. Atwell ('18) found in the rabbit that the lateral lobes, giving rise primarily to the pars tuberalis, do not form a cortex for the entire anterior lobe and that the localized anterior cortex which appears does not persist. In the rabbit, accordingly, it appears unlikely that the segregation of cells in the anterior lobe proper can be related directly to the embryonic history of these regions. The restricted distribution of acidophiles may be related to the functional organization of the gland. In the cat the embryological evidence is not so clear. However, in describing the embryology of the pituitary in man, Tilney ('36) recognized two distinct subdivisions of the pars distalis, an anterior medullary core and a cortical zone which almost surrounds this core. He found that the basis for this subdivision is laid down in early embryonic life and is determined by the appearance of the two lateral diverticula associated with the development of the pars tuberalis. The tips of these processes give rise to the pars tuberalis while the proximal portions form a broad zone about the medial (medullary) portion of the gland which is derived from the median portion of Rathke's pouch. Early in the course of development a deep groove running along either side of the dorsum of the original mouth pouch establishes a boundary between the median and lateral portions of the pars distalis. The subsequent ingrowth of blood vessels and connective tissue trabeculae also emphasize this subdivision of the pars distalis.

Some vestiges of this embryonic history are still evident in the adult human gland and appear to be reflected slightly in the distribution of the types of cells. Rasmussen ('38) finds that the acidophiles tend to concentrate in a large area located somewhat posteriorly in each lateral half of the pars distalis, leaving an area near the mid-sagittal plane and an anterior and a marginal zone where the acidophiles are less numerous and chromophobes and basophiles predominate. There is a tendency for some basophiles to accumulate about two prominent vascular connective tissue trabeculae that extend into the pars distalis. These in turn are usually surrounded by a distinct acidophilic area.

Since neither the morphological or physiological significance of the zona tuberalis is known, it seemed advisable to extend the observations on the topographical relationships of the cells of the anterior pituitary to other mammals, and preferably to those in which the development of the gland, including the differentiation of the pars tuberalis, had been adequately treated. The present study is based on five complete pituitaries from two male and three female opossums. They were obtained through the courtesy of Dr. T. B. Thomas. These pituitaries present several interesting morphological features and some interpretation of the adult condition is possible owing to the comprehensive description of the development of the marsupial pituitary by Parker ('17).

## MATERIALS AND METHODS

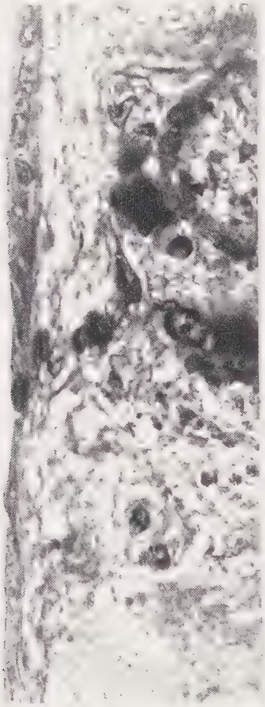
The pituitaries which lie deep in sphenoid bone were freed by dissection and removed with a small portion of the brain attached.

They were fixed in formalin-corrosive sublimate and stained by a modification of Heidenhain's azan method (Dawson and Friedgood, '38). Complete serial sections 4 μ thick were made of all glands. Two glands (one male and one female) were sectioned sagittally, the others were cut frontally.

## DESCRIPTION

The usual four components, infundibular process (posterior lobe), pars intermedia, pars distalis and pars tuberalis are readily recognized. The topography, relative proportions and relationships of the several parts are represented diagrammatically in figures 3 to 8. The body of the gland is attached to the brain by a relatively slender stalk which is occupied by a narrow infundibular recess. The infundibular process itself is solid and is deeply embedded in the pars distalis occupying an almost central position and extending anteroventrally. Except for a small posterodorsal region it is completely invested by a thin pars intermedia which in turn is clearly separated from the pars distalis by a conspicuous residual lumen. At its most ventral extent the lumen is almost in contact with the capsule of the pars distalis being bounded by only a thin layer of epithelium whose cells rarely exhibit any granular differentiation (fig. 3).

The pars intermedia, while extensive and investing the infundibular process, consists of but a single layer of epithelium, although in oblique sections it appears more complex. The height of the epithelium may vary regionally in individual pituitaries. In its maximum development the epithelium consists of tall columnar cells, locally distorted by the presence of large, spherical, lightly-basophilic granular cells. The granular cells are seldom clustered, but are scattered at random throughout the pars intermedia (fig. 2). The free ends of the columnar cells are occasionally ciliated, especially in regions where the granular cells occur. However, for the most part the epithelium is low and flattened, and may even appear squamous, with no evidence of granular differentiation or ciliation (fig. 1). No colloid was seen in the adjoining residual lumen. There was little evidence of accumulation of any secretion in this space.



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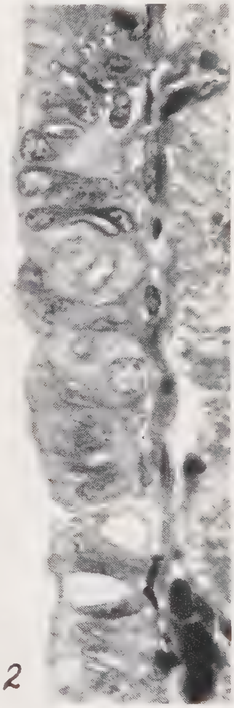

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Fig. 1 Photomicrograph showing the low, flattened epithelium of the pars intermedia (left) and the adjoining tissue of the infundibular process (right).

Fig. 2 Photomicrograph showing a region of the pars intermedia composed of tall columnar cells and isolated granular cells.

The pars distalis is characterized by the marked restriction in the distribution of the acidophile cells. In the region anterior to the embedded posterior lobe, basophiles and chromophobes predominate. Scattered clusters of cells reacting with azocarmine are also seen in this region of the pituitaries of both males and females. It is possible they may be identical with similarly stained cells described for the rabbit and cat, but the evidence at present is inconclusive (Dawson and Friedgood, '38, a, b; Friedgood and Dawson, '38). In the regions lateral and posterolateral to the infundibular process, acidophiles reacting with orange G are numerically dominant but both chromophobes and basophiles are also present in considerable numbers. These two regions are not sharply delimited from one another, but merge more or less gradually in a zone of transition. The general features of this limitation of distribution of certain cell types was also recognized by Tilney ('11).

The pars tuberalis in the adult opossum is poorly developed and shows no tendency to spread into the anterior portion of the pars distalis. It is sharply segregated to the region of the stalk and adjoining surface of the brain. The typical vesicular appearance is rarely attained and little colloid is present. However, it is morphologically continuous with the anterior region of the pars distalis and it seems possible therefore to homologize the portion of the anterior lobe proper lacking characteristic acidophiles with the similar region in the pituitary of the rabbit and cat, i.e., the zona tuberalis. The further question as to the relation of the subdivision of the pars distalis into two zones to the developmental history of this portion of the gland cannot be answered directly by evidence from the opossum.

## DISCUSSION

The descriptions of the pituitary glands of several other marsupials by Parker ('17) indicate that, in general, the pituitary glands of marsupials are morphologically quite similar. Accordingly the salient features of the development of the marsupial pituitary as given by Parker will be summarized.

In the marsupials Trichosurus vulpecula and Phascolarctos cinereus (Parker, '17) Rathke's pouch is early subdivided, in the portion behind the narrowed duct, by a pair of horizontal constrictions. The two lobes thus formed have been designated as a proximal (nearest the opening of the pouch) and distal lobe (nearest to the diencephalic floor). The constrictions disappear at the point where the hypophyseal duct opens so that the anterior open section of the pouch is undivided. The distal lobe becomes large and its walls locally thickened, later giving rise to the pars distalis and pars intermedia. The proximal lobe remains thin-walled and gives rise chiefly to the pars tuberalis.

The wall of the anterior half of the distal lobe thickens rapidly so that the extreme end becomes solid and the lumen progressively reduced while posteriorly the lumen remains large with a thin posterodorsal roof and thick sides. The posterodorsal wall represents the primordium of the pars intermedia. Unlike the rest of the distal lobe, it never thickens markedly, but on the contrary as development proceeds becomes reduced to a thin and very regular layer of epithelium. The infundibular process as it grows downward indents it from above and during the growth of the hypophysis as a whole the pars intermedia spreads around it in all directions so that it comes to cover the infundibular process on its anterior and lateral, and to a certain and variable extent on its posterior surfaces. This envelopment of the infundibular process by the pars intermedia is associated with the development from the thickened side walls of the distal lobe of a pair of large dorsolateral outgrowths which contain narrow cleft-like extensions of the lumen. Connective tissue and capillaries supplying the pars distalis enter by ventral clefts which delimit the lateral wing-like processes from the thickened so-called median lobe.

The proximal lobe remains hollow and thin-walled with its lumen continuous with that of the distal lobe. It becomes drawn out laterally and early becomes crescentic. Its lateral borders curve up toward the dorsal side of the pars buccalis. These wing-like structures are prolonged anteriorly considerably beyond the main body of the proximal lobe. In subsequent stages the median portion of this lobe becomes greatly reduced, loses its lumen, and fuses with the ventral surface of the distal lobe, while the enlarged lateral portions grow up toward the brain floor. Finally, they lose their lumina, branch and form a collection of flattened tubules which, increasing greatly in number, extend first anteriorly to meet and fuse in the mid-line in front of the infundibular stalk while they later penetrate to the caudal side of that structure and thus come to form a complete ring around it—the pars tuberalis.

In all cases in which acidophilic cells of the pars distalis could be observed they were most numerous in the posterior portion. From the description it becomes apparent that in the Australian marsupials the portion of the pars distalis containing the acidophiles is derived from the posterior half of the so-called distal lobe of Parker, chiefly from the pair of large dorsolateral outgrowths which, with the extensions of the pars intermedia, envelop the posterior lobe. In the adult opossum these outgrowths meet posterior to the infundibular process but do not fuse (figs. 5 and 6).

The anterior zone of the pars distalis of the opossum, characterized by the restriction of acidophiles, would appear to be derived primarily from the thickened anterior wall of the distal lobe of Parker with a possible small contribution from the medial portion of the proximal lobe of Parker which becomes reduced and fused with the anterior surface of the distal lobe. Accordingly, it seems impossible to regard the two subdivisions of the pars distalis in the adult opossum as direct derivatives of two separate embryonic zones, one of which gives rise to the medullary portion of the pars distalis, while the other forms the cortex of the pars distalis and the pars tuberalis (Tilney, '36). However, Tilney ('37) feels that the identification of such a cortical area holds good for most of the reptiles and all the mammals which he had had the opportunity to investigate.

In many instances the subdivision of the pars distalis on the basis of the restricted distribution of certain cells is clearly seen as in the rabbit and cat (Dawson, '37). However, in these cases, the anterior medial region (regarded as homologous with the medullary portion by Tilney) is more intimately associated, in the adult gland, with the pars tuberalis than are the posterolateral regions which Tilney believes to be cortical and derived from the lateral processes.

Such regional localizations have been recognized in many other mammals, but in only a few instances have their distribution in relation to the pars tuberalis or other regions of the pituitary complex been studied in any detail. The distribution patterns of the basophiles have been considered by several investigators (Soós, '34, horse, fox, cat, deer; Spaul and Howes, '30, ox; Warbritton and MacKenzie, '37, ewe; Chadwick, '36 and Kirkman, '37, guinea pig). More recently Fawcett ('38) has plotted their distribution in the guinea pig and finds a general correspondence with the condition in the rabbit and cat, but the zone is much more broken up by invasions of typical pars anterior tissue.

In the chicken pituitary (Rahn, '38; Painter, '38) the pars distalis is also subdivided into two distinct zones, anterior and posterior, which have been designated by Rahn as the cephalic and caudal lobes. This cytological differentiation into two lobes is complete at 18 days incubation. The caudal lobe represents typical pars anterior tissue, while the cephalic lobe is characterized by more faintly staining cells which react atypically with the usual methods of differentiation. The atypically staining cephalic lobe is intimately associated with the pars tuberalis, but the complete details of the morphogenetic relationship between these two portions of the epithelial complex have not been published. A similar segregation of the cells of the pars distalis also occurs in the duck (Painter, '38).

The situation in Amblystoma, however, appears to be quite different. Here Roofe ('37) recognizes a pars subdistalis made up of densely stained acidophiles which are constantly grouped in the ventromedial portion of the pars distalis, while the cells of the remainder of the gland are chiefly basophiles. Other regional localizations of cells of special types are also encountered in the fishes forming the 'Übergangsteil' of

Stendell. This localization Tilney ('37) regards as homologous with the cortical zone of the higher vertebrates.

At the present time it appears impossible to make any evaluation of the significance of the region of the pars distalis which has been designated the zona tuberalis. Final solution must wait until more careful studies of the morphogenesis of the pituitary gland have been made, using selective staining methods throughout, so that the developmental history can be directly correlated with the final cytological patterns of distribution of cell types. It may be that these cellular localizations are not evidences of morphogenetic history, but are morphological expressions of functional activity determined by such extrinsic factors as blood supply and innervation.

The extreme simplicity of the pars intermedia and pars tuberalis are other interesting features of the opossum pituitary. The cells of the pars intermedia usually give little evidence of differentiation although in some individuals scattered granular cells are present. In general the same holds true for the pars tuberalis. These conditions in the opossum coupled with the complete absence of the tuberalis in the sloths and anteaters and the lack of a pars intermedia in the whale and armadillo (Wislocki, '38) further emphasize the observation of Wislocki that the most constantly developed components of the pituitary complex are the infundibular process and the pars distalis.

## SUMMARY

The opossum pituitary consists of the four usual components, infundibular process, pars intermedia, pars distalis and pars tuberalis. The infundibular process is solid and deeply embedded in the pars distalis. The pars intermedia is thin and extensive and almost completely invests the infundibular process. The intermedia consists of a single layer of epithelium which is frequently low, flattened and undifferentiated. In certain regions the epithelial cells are tall, columnar and sometimes ciliated, and scattered, spherical,

granular cells may be present. A persistent residual lumen completely separates the pars intermedia from the pars distalis.

The portion of the pars distalis anterior to the infundibular process is composed almost entirely of chromophobic and basophilic cells with scattered clusters of cells which stain with azocarmine. The acidophiles, reacting with orange G, are confined to the more lateral and posterior portions of the gland. The anterior subdivision of the distalis is homologized with the zona tuberalis recognized in the cat and rabbit. The pars tuberalis proper of the opossum is reduced and exhibits little differentiation, but is morphologically continuous with the zona tuberalis of the pars distalis. The possible significance of the regional restriction of distribution of cell types in the pars distalis is discussed.

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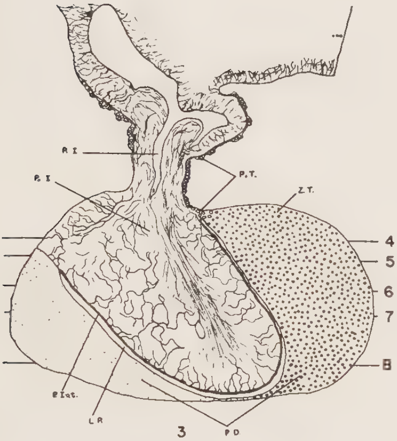
## PLATE 1

### EXPLANATION OF FIGURES

All figures were outlined at the same magnification and the distribution of the various parts indicated as follows: pars distalis (P.D.), fine stipple; pars intermedia (P.Int.), solid black; pars tuberalis proper (P.T.), large open circles; zona tuberalis (Z.T.), large solid dots; infundibular process (P.I.), irregular lines; infundibular recess, R.I., residual lumen, L.R.

3 Sagittal section of the opossum pituitary showing the topography, relative proportions and relationships of the several parts. The numbered lines (4 to 8) indicate the approximate levels from which the frontal sections (figs. 4 to 8) were drawn.

4 to 8 Successive frontal sections from the levels indicated in figure 3.



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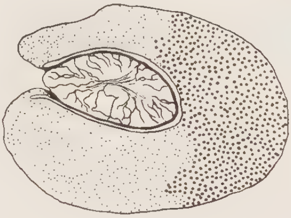

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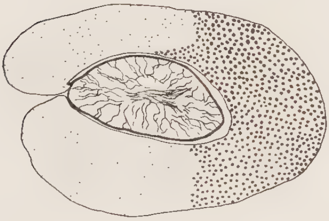

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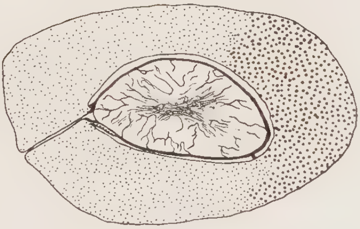

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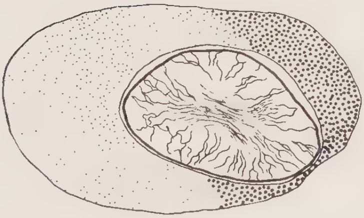

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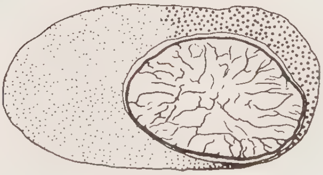

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# A QUANTITATIVE STUDY OF THE INFLUENCE OF THE OVARIAN HORMONES ON HYPERPLASIA BY MITOSIS IN THE RABBIT UTERUS IN EARLY PREGNANCY[1]

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TWO FIGURES

## INTRODUCTION

The role of the smooth muscle in the growth of the uterus during pregnancy was first reported by Koelliker in 1849. He pointed out two distinct factors which caused an increase of uterine muscle: first, the enlargement of existing muscle cells; second, the formation of new muscle cells from young round cells. Hypertrophy, however, as a mode of uterine enlargement has tended to overshadow the role of hyperplasia.

Since Koelliker's time, numerous investigators have expressed without conclusive evidence a belief that hyperplasia is a factor in the growth of the pregnant uterus. Among such investigators are Kilian (1849), Heschl (1852), Luschka (1864), Veit (1885), Sanger (1887), Nagel (1896), and Moraller, Hoehl and Meyer ('12). Then, in 1922, Amati reported hyperplasia of the uterine muscle of the rabbit which occurs through mitotic division of the muscle cells during the second to the fourth days of pregnancy. He could not find these mitoses from the fifth day on, and did not speak of muscle cells differentiating from connective tissue cells.

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[1] The author wishes to make a correction in the abstract of this paper as presented at the meeting of the American Association of Anatomists, April, 1938 (Crandall, Anat. Rec., vol. 70, suppl. 3, p. 18). In this abstract the figures which express the number of mitoses per square centimeter are one-tenth of the correct figure.

Froböse ('32) confirmed Amati's report that in the pregnant rabbit uterus muscle cells increase in numbers by mitotic division, but claimed that the mitoses could be found throughout the entire gestational period; and he further reported that new muscle cells develop from connective tissue cells. Although mitotic division of muscle cells in the human pregnant uterus has been reported (Grynfelt), the general opinion is that throughout the period of gestation numerous new muscle cells are differentiated from connective tissue cells in the human uterus and not by mitosis of existing muscle cells (Grynfelt, '25; Stieve, '26 and '29; Keiffer, '28; Valle, '35).

Not much work has been done in this field on the rat or mouse. In 1930 Gander reported that hyperplasia of the uterine muscle in the pregnant rat and mouse occurs only through mitosis of existing muscle cells. Five years later Froböse ('35) claimed that in the mouse, pregnancy hyperplasia of the uterine muscle is a combination of existing muscle cell mitosis and differentiation of new muscle cells from connective tissue cells.

Other studies have shown that muscle hyperplasia in the nonpregnant uterus occurs in a like manner to that described above in the pregnant uterus. Connective tissue cell differentiation into new uterine muscle cells occurs in the menstrual cycle of humans, according to Stieve ('26), and Joachimovits ('28); and in that of monkeys (Joachimovits, '28). Muscle cell mitoses have been demonstrated in the regeneration following uterine wounds, by Ritschl (1887) in rabbits, Busachi (1887) in rabbits, guinea pigs, and dogs, Walsh and Loeb ('17) in guinea pigs, Berry ('20) in the human, rabbit, and guinea pig, and McIntyre ('23) in the human. Muscle cell mitoses have been shown in the uteri of infantile mice treated with pregnancy urine (Fischer-Wasels and Büngler, '31), in the uteri of pituitary-treated immature rats (Fischer-Wasels, '32; and Gander, '30), and in the uteri of non-castrated infantile rats under the influence of estrin (and their own corpora lutea) (Gander, '30). Mitoses have been found in the uterine musculature of ovariectomized mice and rats treated with large doses of estrin and in normal mice and rats treated with prephysin or with pregnant mares' serum (Allen, Smith and Gardner, '37). Mitoses have also been observed in the muscle cells of uteri of ovariectomized rabbits which had been distended with paraffin pellets (Allen, Smith and Reynolds, '37).

In view of the foregoing considerations, it appears certain that hyperplasia of the uterine musculature is an important factor in the growth of the uterus at certain times, including pregnancy. That it has not been accepted as such in pregnancy growth is due chiefly to the lack of a quantitative comparison of the amount of growth due to hyperplasia and the amount due to hypertrophy.

The following work was undertaken with a twofold purpose; first, to establish on a quantitative basis the extent to which hyperplasia by mitotic division of the uterine muscle cells of the circular layer and the associated connective tissue cells in the same area occurs in the first week of pregnancy in the rabbit; and second, to determine the relative role of the crystalline hormones estrin and progesterone in these processes. No attempt was made to determine whether connective tissue cells differentiate into new smooth muscle cells nor was an attempt made to classify the dividing connective tissue cells into groups, as fibrocytes, myoblasts, histiocytes, etc.

## EXPERIMENTAL WORK

For these experiments female rabbits of heterogeneous stock, age, body weight, and previous sexual history were used. It is quite probable that the quantitative determinations would have been relatively more significant if rabbits of homogeneous types could have been used. All rabbits were isolated for at least 3 weeks previous to use, and all were proven sexually mature by ovulation following coitus. Seven series of experiments were performed (table 1):

TABLE 1

| EXPERIMENT | DAYS AFTER MATING | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1st[1] | 2nd | 3rd | 4th | 5th | 6th | 7th |
| Normal pregnancy | | 15—O | 15—A | 13—O | 13—A | 14—O | 14—A |
| | | 37—O | 37—A | 39—O | 39—A | | |
| | | 38—O | 38—A | | | | |
| Untreated castrates | 19—C | 19—O | 19—A | | | | |
| | 35—C | 35—O | 35—A | | | | |
| | 36—C | 36—O | 36—A | | | | |
| | 18—C | | | 18—O | 18—A | | |
| | L-1—C | | | L-1—O, A | | | |
| | L-4—C | | | L-4—O, A | | | |
| | 20—C | | | | | 20—O | 20—A |
| 50 I.U. estrin daily | 24—C | 24—O | 24—A | | | | |
| | 42—C | 42—O | 42—A | | | | |
| | 43—C | 43—O | 43—A | | | | |
| | 25—C | | | 25—O | 25—A | | |
| | L-2—C | | | L-2—O, A | | | |
| | L-5—C | | | L-5—O, A | | | |
| | 28—C | | | 28—A | | | |
| 500 I.U. estrin daily | 26—C | 26—O | 26—A | | | | |
| | 27—C | | | 27—O | 27—A | | |
| | 44—C | | | 44—O | 44—A | | |
| | 45—C | | | | | 45—O | 45—A |
| 0.2 mg. of progesterone daily | 10—C | 10—O | 10—A | | | | |
| | 40—C | 40—O | | 40—A | | | |
| | 41—C | 41—O | | 41—A | | | |
| | 9—C | | | 9—O | 9—A | | |
| | 32—C | | 32—O, A | | | | |
| | 33—C | | 33—O, A | | | | |
| | 16—C | | | | | 16—O | 16—A |
| | 3—C | | | | | 3—O, A | |
| 0.5 mg. of progesterone daily | 12—C | 12—O | 12—A | | | | |
| | 11—C | | | 11—O | 11—A | | |
| | L-3—C | | | L-3—O, A | | | |
| | L-6—C | | | L-6—O, A | | | |
| | | | | x—616 | | | |
| | 17—C | | | | | 17—O | 17—A |

C = Test piece of left horn taken at time of castration.
O = Left horn of uterus.
A = Right horn of uterus.
L = Lloyd's experiments ('37).

[1] Twelve additional uteri of the first day, not listed here = total of 43.

1. Forty-three rabbits were mated and castrated 18 to 24 hours after coitus. At this time ovulation was determined to have taken place in every case, and a control piece of uterus was taken from near the middle of the left horn. These forty-three control tissues are of uteri of rabbits 18 to 24 hours after mating, unmodified by experimental procedure, for in no case was hormonal treatment begun previous to castration and the removal of this tissue. Twelve of these rabbits were subsequently used in experiments other than those of this paper. The remaining thirty-one are listed in table 1, in the column '1st day after mating' according to the experimental group in which they were used.

2. Six rabbits were mated and not castrated, i.e., they were either pregnant or pseudopregnant ('normal pregnancy,' table 1).

3. Seven rabbits were mated, castrated 18 to 24 hours after coitus, and not given any hormonal treatment ('untreated castrates,' table 1).

4. Seven rabbits were mated, castrated 18 to 24 hours after coitus, and given daily subcutaneous injections of 50 I.U. of crystalline estrin in 0.25 cc. of sesame oil beginning immediately after castration and ending 24 hours before autopsy (except the two rabbits kindly contributed by Lloyd from his experiments ('37) which received 50 I.U. of estrin in the form of amniotin daily) ('50 I.U. estrin daily,' table 1).

5. Four rabbits were treated in a similar manner to those of group 4 except that the dosage was 500 I.U. of crystalline estrin in 0.1 cc. of sesame oil ('500 I.U. estrin daily,' table 1).

6. Eight rabbits were mated, castrated 18 to 24 hours after coitus, and given daily subcutaneous injections of 0.2 mg. of crystalline progesterone in 0.2 cc. of sesame oil beginning immediately after castration and ending 24 hours before autopsy (0.2 mg. progesterone daily,' table 1).

7. Six rabbits were treated in a similar manner to those of group 6 except that the dosage was 0.5 mg. of crystalline progesterone in 0.2 cc. sesame oil. A further exception is rabbit x-616. This rabbit, from W. M. Allen's experiments,

received 1.5 mg. of crystalline progesterone in four doses over 2 days ('0.5 mg. progesterone daily,' table 1).

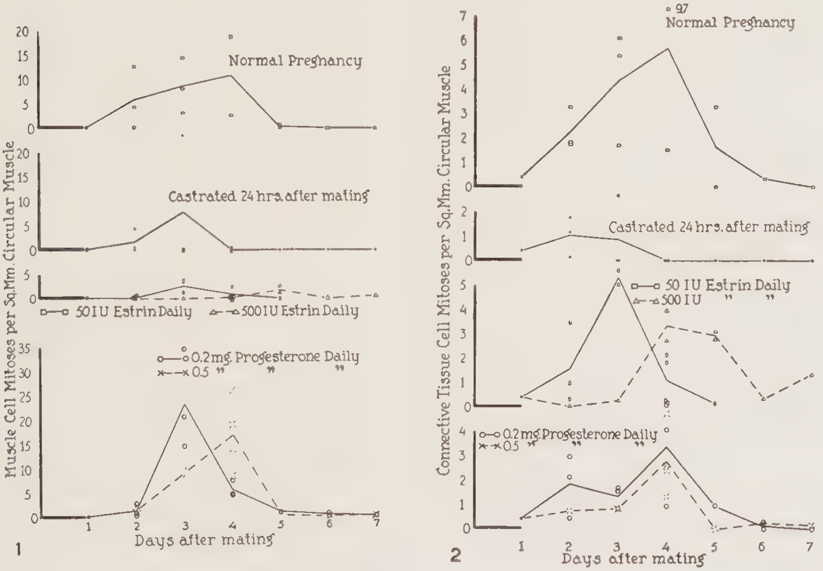
With a few exceptions, the left horn of the uterus was removed at operation; and the right horn, at autopsy 24 hours later (table 1). The tissues were fixed in Bouin's fluid, paraffin-embedded, cut at 8 μ, and stained by Crossmon's ('37) modification of Mallory's connective tissue stain. At least three cross sections of each tissue, from near the middle of the uterine horn, were studied under high power and the nuclei undergoing mitosis in the circular muscle layer were counted. Any stage of mitosis which could be recognized with certainty was included in the count. The average of the counts for each tissue was then related to the area of the circular muscle in cross section of the tissue, as determined by planimeter measurement of a projection of the cross section, in terms of mitoses per square millimeter.

Colchicine was not used in these experiments because mitotic activity was clearly in evidence without the use of the alkaloid, because the dosage and optimum time of response to the drug has not been worked out for the rabbit uterus, and because the action of the drug has not been definitely settled.

## RESULTS AND DISCUSSION

*1. Mitoses 18 to 24 hours after coitus.* A study of control pieces taken from the left horn of the uterus of forty-three rabbits at time of castration, 18 to 24 hours after coitus, shows no mitoses in either muscle or connective tissue cells in 58% of the cases; no muscle cell mitoses in 72%, and no connective tissue cell mitoses in 67% of the cases. The highest counts for a single cross section were nine for muscle cell and twenty-eight for connective tissue cell mitoses. Counts of mitotic figures of three cross sections from each of the forty-three rabbit uteri give an average of 0.17 muscle cell mitoses and 0.41 connective tissue cell mitoses per square millimeter of circular muscle. These results show that hyperplasia by mitosis of muscle and connective tissue cells is just beginning in the circular muscle layer of the rabbit uterus 24 hours after mating (figs. 1, 2).

2. *Mitotic figures in the first week of pregnancy.* A study of uterine tissues taken from six pregnant rabbits shows a high incidence of circular muscle cell mitoses (3 to 19 mitoses per square millimeter) in two out of three uteri on the second day, three out of three on the third day, and two out of two on the fourth day after mating. One uterus on the fifth day showed a few mitoses, another showed none. No muscle cell mitoses were observed in the single uteri examined on the sixth and seventh days after mating (fig. 1).



Figures 1 and 2

The connective tissue cells associated with the circular muscle showed a high incidence of mitoses (1.5 to 9.8 per square millimeter) in these rabbits on the second, third, fourth and possibly the fifth day after mating, and practically no mitoses later (fig. 2).

In the first week of pregnancy there is a wave of mitoses of both types of cells in the circular muscle layer of the rabbit uterus which begins 24 hours after coitus and is practically over by the fifth day after mating. This finding is in accord with the findings of Froböse ('32) and Amati ('22).

*3. Mitotic figures in rabbits castrated 18 to 24 hours after mating.* a. Untreated. Two of the three uteri examined on the second day after coitus showed muscle cell mitoses (0.65 and 4.38 per square millimeter). The third had no muscle cell mitoses. Two of the three examined on the third day showed no muscle cell mitoses; but a third, unaccountably, had a count of 23.47 per square millimeter. Three uteri on the fourth day and one on each of the fifth, sixth and seventh days after mating showed no mitoses (fig. 1).

The connective tissue associated with the circular muscle in the uteri of these rabbits shows a similar rate of mitosis as the muscle. There were mitoses in all three uteri of the second day (0.14 to 1.77 per square millimeter), no mitoses in uteri of the third day except in that uterus which had a high muscle cell mitosis count (2.65 per square millimeter for connective tissue cell mitoses), and no mitoses in the three uteri of the fourth day nor in the uteri of the fifth, sixth or seventh days (fig. 2).

In view of the rest of the experimental work the one specimen among these untreated castrate animals which exhibited a high mitotic count on the third day seems out of place. That is to say, it has a mitotic count more like that of a progesterone-treated rabbit (23.47 muscle cell mitoses per square millimeter). However, the ovaries were completely removed from this rabbit at the usual time; and the epithelium shows no proliferation, a fact which indicates castration and absence of progesterone (Lloyd, '37). It would seem better to omit this uterus, as unexplainable, at present, and to plot the curve on a basis of the other two uteri of the third day. Except for high count in this one instance, the mitotic incidence is low and practically confined to the second day after mating in these untreated castrates. In contrast to this are the pregnant, estrin-treated, and progesterone-treated rabbits with both a higher rate and a wider chronological spread of mitoses. These results indicate that the mitoses observed in the untreated castrates were due to ovarian hormone elaborated prior to castration; that with removal of the ovaries, the source of estrin and progesterone is removed; and finally, that these hormones play an important role in the hyperplasia by stimulating mitotic division of muscle and connective tissue cells in the circular muscle layer of the rabbit uterus.

b. Estrin-treated following castration. In the uteri of these rabbits, muscle cell mitoses are conspicuously few. On the second day after coitus there were no muscle cell mitoses in the circular muscle of the rabbit receiving 500 I.U. daily, nor in that of two of three rabbits receiving 50 I.U. daily, and only 0.40 per square millimeter, in the other rabbit receiving 50 I.U. daily. On the third day there were no muscle cell mitoses in the rabbit receiving 500 I.U. daily and but 1.34 and 3.72 per square millimeter in the two receiving 50 I.U. daily. On the fourth day the four uteri of rabbits receiving 50 I.U. daily showed counts of 0, 0.12, 0.65 and 2.33; those of the two receiving 500 I.U. daily, 0.11 and 0.39 muscle cell mitoses per square millimeter. On the fifth day the uterus of the single rabbit receiving 50 I.U. daily showed no mitoses; the uteri of two receiving 500 I.U. daily, 0.96 and 2.54 muscle cell mitoses per square millimeter. A single uterus of a rabbit receiving 500 I.U. daily gave counts of 0.08 and 0.60 muscle cell mitoses per square millimeter on the sixth and seventh days, respectively (fig. 1).

In estrin-treated rabbits the incidence of muscle cell mitoses is low and is delayed by larger daily doses of estrin. It is, however, of similar magnitude in respect to both numbers of mitoses and duration of mitotic activity whether the daily estrin dosage be large or small. The minimal effective dose was not ascertained.

The mitotic activity of connective tissue cells in these estrin-treated rabbits stands in marked contrast to that of the muscle cells. Already under way 24 hours after coitus, the connective tissue cell mitoses in the circular muscle area of the uterus of rabbits receiving 50 I.U. daily have climbed by the second day to an average of 1.59 per square millimeter (three uteri: 0.30, 0.99, 3.47); by the third day to an average of 5.30 per square millimeter (two uteri: 5.00 and 5.60); and are still continuing on the fourth and fifth days, though at decreasing levels (0.22, 0.23, 1.82 and 2.14 for the fourth and 0.19 for the fifth days). The daily dosage of 500 I.U. of estrin apparently delays the response (single uteri on the second and third days showing counts of 0.03 and 0.28, respectively), but attains a response comparable to the maximum response to the 50 I.U. daily dosage on the fourth and fifth days after mating (2.72 and 3.94 on the fourth day and 2.77 and 3.10 mitoses per square millimeter on the fifth day). The counts of 0.33 and 1.34 connective tissue cell mitoses per square millimeter on the sixth and seventh days, respectively, in the uterus of a rabbit receiving 500 I.U. estrin daily, show that this connective tissue cell mitotic activity has not yet ceased but is declining (fig. 2).

Estrin apparently plays a role in the hyperplasia brought about by mitotic division of connective tissue cells associated with the circular muscle in the rabbit uterus during the first week of pregnancy, and exerts little influence on the mitotic activity of the muscle cells. How much of the influence of estrin on the mitotic activity of the cells can be attributed to its property of inducing hyperemia and increasing the metabolic rate of the uterine tissue (MacLeod and Reynolds, '38) is not known, but it may be considerable. The extremely high incidence of connective tissue cell mitoses specifically stimulated by estrin administration offers factual support for the theory of hormonal origin of uterine fibroids suggested by Witherspoon, and others.

c. Progesterone-treated following castration. All of the uteri of these rabbits show both muscle cell mitoses and connective tissue cell mitoses, with the exception of one rabbit receiving 0.2 mg. daily, which had one horn removed 6 days after mating and the other on the seventh. In neither horn of this rabbit's uterus were mitoses found. The uterus also showed no proliferation and is therefore questionable. One rabbit receiving 0.5 mg. daily showed no connective tissue cell mitoses on the fifth day after mating. Irrespective of daily dosage the counts of both muscle and connective tissue cell mitoses on each of the days were of the same range. The counts of muscle cell mitoses in the uteri of rabbits receiving 0.2 mg. daily were a little higher on the third and a little lower on the fourth day after coitus than those of rabbits receiving 0.5 mg. daily. The counts of connective tissue cell mitoses were generally a little higher for the rabbits receiving 0.2 mg. than for those receiving 0.5 mg. daily. The counts of muscle cell mitoses were as follows: second day, 0.44, 0.85, 2.81 and 1.48; third day, 34.72, 20.71, 14.65 and 9.26; fourth day, 4.53, 4.77, 7.50, 8.54, 14.04, 19.14 and 26.23; fifth day, 0.97 and 0.26; sixth day, 0, 0.13 and 0.61; and on the seventh day, 0 and 0.18 mitoses per square millimeter (fig. 1). The counts of connective tissue cell mitoses were: second day, 0.44, 2.13, 2.98 and 0.74; third day, 0.83, 1.57, 1.69 and 0.84; fourth day, 0.94, 4.09, 5.13, 1.35, 2.41, 2.63 and 4.75; fifth day, 0.97 and 0; sixth day 0, 0.30 and 0.25; and on the seventh day after coitus, 0 and 0.18 mitoses per square millimeter (fig. 2).

It is clear therefore that progesterone exerts an influence on both muscle and connective tissue cell mitosis, but its effect is greater upon muscle than on connective tissue cells. It is not, however, quite as influential as estrin in causing connective tissue cell mitosis, although it is definitely more influential in causing muscle cell mitosis than estrin. The hyperplasia which occurs by mitotic activity of the muscle and connective tissue cells in the circular muscle layer of the rabbit uterus during the first week of pregnancy is dependent upon the action of both estrin and progesterone.

*4. Importance of hypertrophy in uterine growth during pregnancy.* It is a well-established fact that the increase in uterine tissue during pregnancy is attributable mainly to the myometrium, and that the proportional increase of the myometrium is greater even than that of the entire uterus (Stieve, '29; Markee, Wells and Hinsey, '36, and others). Therefore it is interesting to speculate, on the basis of existing data, what the role of hypertrophy actually may be.

One can compute from the following data of Markee, Wells and Hinsey ('36) the increase of the volume of the myometrium in the uterus of the rabbit during pregnancy:

*Mean values*

| | *Normal animals*<br>*cc.* | *26 days pregnant* |
|---|---|---|
| Longitudinal muscle, right horn | 0.135 | |
| Longitudinal muscle, left horn | 0.138 | 3.779 |
| Connective tissue between the two muscular layers, right horn | 0.111 | |
| Connective tissue between the two muscular layers, left horn | 0.108 | 2.352 |
| Circular muscle, right horn | 0.181 | |
| Circular muscle, left horn | 0.183 | 5.952 |

Doubling the values for the left horn in the pregnant rabbits because the right horn contained no fetuses, we find the values of the total myometrium to be 0.856 in the normal animal and 24.166 in the animal pregnant for 26 days. This indicates an increase of myometrium about twenty-eight times.

From the data of Froböse ('32) we can compute the increase in size of the muscle cells in the uterus of the rabbit during pregnancy:

| | *Muscle cell length* | *Muscle cell width* |
|---|---|---|
| Adult non-pregnant rabbit | 55 to 70 μ | 3.5 to 4 μ |
| 30 days pregnant rabbit | 113 to 135 μ | 4 to 8 μ |

Assuming that the muscle cells are spindle-shaped, we find values of muscle cell volume (after the formula $3.1416 \times r^2 \times L/3$) to be 233 $\mu^3$ for the non-pregnant rabbit and 1168.7 $\mu^3$ for the 30 days pregnant rabbit. This indicates a 5.2-fold increase in muscle cell volume.

On this basis we find that hypertrophy of the muscle cells accounts for only one-sixth to one-fifth of the volume increase of the myometrium; and is therefore only one of a number of factors (connective tissue cell hypertrophy, increase of blood vessel volume, hyperplasia, etc.) which take part.

## SUMMARY

1. By a quantitative method the incidence of mitoses in muscle cells and associated connective tissue cells in the circular muscle region of the uterus of the rabbit has been studied during the first week of pregnancy, the first week in untreated rabbits castrated 18 to 24 hours after mating, and the first week in rabbits ovariectomized 18 to 24 hours after coitus and subsequently given 50 I.U. of estrin, 500 I.U. of estrin, 0.2 mg. progesterone, or 0.5 mg. progesterone daily.

2. In the rabbit during the first week of pregnancy there is a hyperplasia of the circular muscle cells in the uterus by means of mitotic division of existing muscle cells which takes place, in the absence of distention, during the first 4 or 5 days after coitus. At the same time there is a hyperplasia, by mitosis, of the connective tissue cells associated with the circular muscle. This increase in the number of cells naturally decreases the amount of hypertrophy necessary subsequently to account for the increase of the myometrium during the period of gestation.

3. Progesterone is more effective than estrin in causing mitotic division of the muscle cells; conversely, estrin is more effective in causing mitosis of the connective tissue cells than is progesterone. The hyperplasia by mitosis in the circular muscle layer of the rabbit uterus during the first week of pregnancy is due to the combined effect of estrin and progesterone.

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# CELLULAR CHANGES IN THE ANTERIOR HYPOPHYSIS DURING THE REPRODUCTIVE CYCLE IN THE FEMALE GUINEA PIG[1]

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FIVE FIGURES

In 1929 Smith and Engle and in 1931 Wolfe reported that the anterior hypophysis exhibits cyclic variations in its capacity to stimulate the ovary. Since then efforts have been made to determine if there are not also cyclic variations in the percentage of cell types and the extent of granulation. Presumably such variations would be correlated with variations in physiological activity and a means would be provided for the association of cell types with specific gonadotropic functions.

Several mammalian species have been studied. For the rat conflicting reports are given. Reese ('32) found that the acidophiles are highly granular during metoestrum and degranulated during dioestrum, but that there is no change in the basophiles. Charipper and Haterius ('32) state that the acidophiles are sparse and degranulated during oestrus, and numerous and large in dioestrum; basophiles are prominent during oestrus and small and degranulated in dioestrum. Wolfe and Cleveland ('33) and Wolfe ('35) report that the acidophiles show little change either in relative percentage or granulation during the cycle, but that the basophiles decrease in percentage and show marked degranulation during metoestrum. The results obtained from investigations of the

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guinea pig are also conflicting. Chadwick ('36) found that the acidophiles are lowest in relative percentage during metoestrum and dioestrum and increase during prooestrum and oestrus; the basophiles are lowest in relative percentage during metoestrum and dioestrum, and highest during prooestrum with a decrease during oestrus. Kirkman ('37) reports that the acidophiles are low in relative percentage during metoestrum, highest during dioestrum, lowest during prooestrum, and high again during oestrus; the basophiles are lowest in relative percentage during oestrus and highest during dioestrum. Cleveland and Wolfe ('33) report that in the sow the acidophiles become somewhat degranulated and decrease in relative percentage during the lutein phase (metoestrum and dioestrum) and that the basophiles show marked degranulation during oestrus and a slight decrease in percentage during the lutein phase. Chadwick notes that these changes are essentially similar to those in the guinea pig. Observations on the dog (Wolfe, Cleveland and Campbell, '33) indicate that the acidophiles do not change appreciably in percentage or granulation, but the two types of basophiles described become degranulated and decrease significantly in percentage during oestrus and the lutein phase. In the rabbit (Wolfe, Phelps and Cleveland, '34) both acidophiles and basophiles show a decrease in percentage and granulation during pseudopregnancy whether ovulation was induced by injections of anterior pituitary hormone or by sterile copulation. In general, Wolfe and his collaborators feel that the percentage of basophiles is low during the lutein phase and higher at other times. However, when all the work is reviewed, agreement on this point as well as others is not complete.

A favorable opportunity for a re-investigation of the subject in the guinea pig arose during observations on the reproductive cycle of this species. Information bearing on certain factors which might affect the hypophyseal picture was accumulating and a better controlled investigation was possible than had been previously. The time of heat was being identified more accurately, detailed records of cyclic reproductive behavior existed and the ovarian condition was known.

## MATERIALS AND METHODS

Thirty-eight adult female guinea pigs were selected from a colony of not less than 300. Twenty were of known age and all were under continuous day and night observation from one to fifteen cycles. Their care and condition are described elsewhere (Young, Dempsey, Myers and Hagquist, '38).

The animals were killed at different times during the cycle in order to determine if variations in the percentage of cell types are correlated with different phases of the reproductive cycle and with different ovarian conditions, and to study changes in the extent of granulation, the Golgi apparatus and mitochondria. Heat was identified by means of the copulatory response (Young, Dempsey and Myers, '35; Young, '37; and Young, Dempsey, Hagquist and Boling, '37). The hypophyses were removed at definite times with respect to the final heat. The groups listed below were established:

| | *Animals* |
|---|---|
| Beginning of heat | 4 |
| End of heat | 5 |
| Fourth day | 5 |
| Sixth day | 3 |
| Eighth day | 8 |
| Tenth day | 3 |
| Twelfth day | 5 |
| Fifteenth day (prooestrum) | 5 |

The time of death is accurate to within ½ hour for the animals killed at the beginning and end of heat. For those killed on the fourth, sixth, eighth, tenth and twelfth days, the time is accurate within 4.5 hours. Animals in the prooestrous group were killed about the fifteenth day, but never until the vaginal membrane had ruptured and always prior to the expected heat. Four were killed 15 days, 6.5 hours after the beginning of the previous heat, and the fifth, 14 days, 14 hours after the beginning of the previous heat.

The animals were killed by a blow on the back of the head. The hypophyses were removed immediately and fixed. The ovaries, thyroid, adrenals and a portion of the uterus were also removed and fixed in Bouin's fluid.

The hypophyses were fixed by the Nassonov-Kolatschev method. The procedure was slightly modified from that described by Severinghaus ('32). Osmic acid impregnation was done at 36° to 37°C. rather than at 40°, followed by subsequent treatment at 35°. The tissue was embedded in 62° paraffin and serial sections were cut in the sagittal plane. In order to obtain representative sections from several regions of the entire gland, five equally spaced zones were cut at 2 μ, thirty sections to a zone; the alternate six zones were cut at 4 μ, with the same number of sections per zone. The number of 4 μ sections per zone was determined by measuring the whole gland with a vernier caliper before embedding, calculating the number of micra, and laying out the alternate 4 μ and 2 μ zones. This method is essentially the same as that described by Kirkman ('37).

Sections on one slide from each of the 2 μ zones were stained for a study of the mitochondria by the technique used by Severinghaus ('32) for differentiating mitochondria from the acidophilic granules. Sections on a second slide were stained by the Mallory azan method of Heidenhain. This slide was used for studying the Golgi apparatus and for making cell counts. Sections on the third slide were left unstained. All the sections at 4 μ were stained by the Mallory azan method of Heidenhain.

The counting technique was the same as that used by Kirkman ('37). An average of 1444 cells per hypophysis was counted. However, since variations in the percentage of cell types may not express accurately the changes that occur in the hypophysis during the reproductive cycle (Severinghaus, '37), study of the extent of granulation, the Golgi apparatus and mitochondria was deemed advisable.

The condition of the Golgi apparatus and mitochondria was determined by ordinary microscopic examination. The extent of granulation was estimated and expressed for both the acidophiles and basophiles by arbitrary values ranging from 0 to 6+. The granular content was estimated since no satisfactory method for making quantitative determinations has been devised.[2] Throughout the work, in order to obviate subjective factors, the slides were examined at random.

The writer wishes to express his appreciation to Prof. William C. Young for his advice in formulating and completing this investigation, and to Dr. Edward W. Dempsey and Messrs. Richard J. Blandau, John L. Boling and Vincent J. Collins for invaluable help in observing animals. Gratitude is also expressed to Prof. Aura E. Severinghaus for valuable advice regarding some of the material used in this study, and especially to Dr. Hadley Kirkman for a number of photographs, detailed suggestions regarding technique and the loan of several slides used in checking cell counts.

## OBSERVATIONS

*Cell types.* Two types of acidophiles were observed. One, comprising about 12.0% of all the cells, contains a densely staining homogeneous appearing cytoplasm. The nucleus usually is small and condensed, stains a deep blue and frequently contains one or two acidophilic bodies which range in size from tiny granules to structures which almost fill the nucleus. These cells are designated as dark acidophiles. The second type comprises about 35.0% of the cells. The cytoplasm stains a little lighter than that in the dark acidophiles, and is granular. The cells range from those in which the granules are closely packed to those containing relatively few granules. In these degranulated cells a pale blue matrix is frequently seen. The nuclei stain a light blue, are usually larger than those of the dark acidophiles, and more or less vesicular. Small acidophilic bodies are frequently observed, but they are never large. Both types of cells are distributed throughout the gland, but tend to be concentrated in the lateral portions along the periphery. Transitional stages were observed, and although the evidence is not conclusive, the indication is that they are different phases of the acidophilic cell type. In table 1 the percentages of each cell type are shown, but when the graphs (figs. 1 and 4) were prepared, the two cell types were considered as one.

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[2] Severinghaus (personal communication) has made photoelectric determinations of the extent of basophilic and acidophilic granulation in the anterior hypophysis. By this method he can readily differentiate the hypophyses of normal and castrate rats. The method, however, is still impracticable when the granular variations are not marked.

In the acidophile the Golgi is usually small, and seems to adhere to one side, or sometimes to surround the nucleus entirely. In some cells strands of the Golgi apparatus ramify into the cytoplasm and sometimes almost fill the cell. This is especially true of the dark acidophiles, but in these the Golgi apparatus usually has a fragmented appearance. No acidophile was seen in which the Golgi apparatus was entirely separated from the nucleus and located out in the cytoplasm where it is found in the basophile.

Two types of basophiles, comparable to those described by Kirkman ('37) were observed. They tend to be concentrated somewhat toward the pars tuberalis. The dark basophile is characterized by a deep blue cytoplasm which is finely granular. The nuclei usually stain a deep blue, but frequently contain plasmasomes which vary considerably in size. The entire nucleus often takes on the acidophilic stain as though it were filled with plasmasomes. In general the dark basophile is the largest of the parenchymal cells. It comprises about 3.6% of the cells.

The light basophile is similar to the dark basophile except that the cytoplasm stains less intensely and is not as granular. In many of the cells cytoplasmic filaments staining a light blue were observed. The nuclei are deeply basophilic, and only rarely do they contain acidophilic inclusions. Usually the light basophile is comparable in size to the dark basophile, but some are as small as the chromophobes. They represent about 8.0% of the cells. As with the acidophiles, the percentage of each cell type is shown in table 1, but in the preparation of figures 2 and 4 they were considered together.

The structure of the Golgi apparatus in the basophile is characteristic. As a rule, it is large and consists of a complex of anastomosing threads occurring in the cytoplasm at some distance from the more or less eccentrically placed nucleus. In the smaller basophiles of both types, especially during the early part of the cycle, the Golgi apparatus is frequently small and compact, and lies fairly close to, but not in contact with, the nucleus.

The chromophobes, comprising about 41.3% of the cells, are the smallest in size. The cytoplasm is very scanty and stains lightly, if at all. The nucleus is basophilic and usually is more or less vesicular. It occupies most of the cell. Although two types of Golgi apparatus comparable to those in the basophiles and the acidophiles were observed, the structures were not consistent enough to make them a reliable index for classifying most of the chromophobes as acidophilic or basophilic as Severinghaus ('33) did for the rat.

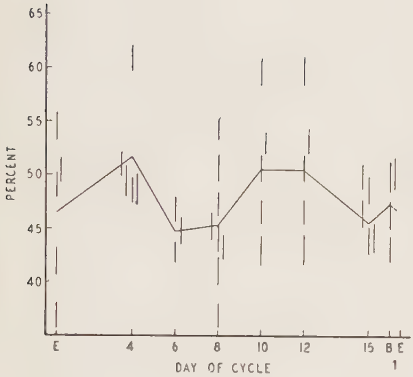
*Changes in the relative percentage of cell types during the reproductive cycle.* Early in the work it was thought that if the secretions of the basophiles or acidophiles are responsible for the sudden appearance of heat, marked changes might be shown in the cell types prior to heat. Or, since the secretions of these cells are assumed to participate in the regulation of cyclic activity, variations in their number might be related to some other phase of the cycle.

The relative percentages of the various cell types at different stages in the cycle are shown in table 1 and figures 1 to 3. The percentage of the different cell types for each animal is at the midpoint on the vertical lines. The length of each line indicates the standard error for that particular percentage. The figure was obtained by using the formula $SE = \sqrt{\frac{pq}{N}}$, where $p =$ per cent, $q = 1-p$, and $N =$ number of cells counted per hypophysis (nomograph 25, Dunlap and Kurtz, Handbook of Statistical Nomographs, Tables and Formulas). The percentages for the animals in each group are arranged vertically to indicate the distribution. The curves represent averages of the results obtained from the animals in each group, but since the variations are so great, the averages are not statistically significant and cannot be used to show a correlation with the

TABLE 1

*The percentage of cell types in the anterior hypophyses of female guinea pigs during the reproductive cycle*

| GROUP | ANIMAL | DARK ACIDOPHILES | LIGHT ACIDOPHILES | TOTAL ACIDOPHILES | DARK BASOPHILES | LIGHT BASOPHILES | TOTAL BASOPHILES | CHROMOPHOBES |
|---|---|---|---|---|---|---|---|---|
| Beginning of heat | 55 | 11.5 | 38.4 | 49.9 | 2.3 | 18.6 | 20.9 | 29.2 |
| | 66 | 21.3 | 29.1 | 50.3 | 2.0 | 10.9 | 12.8 | 36.8 |
| | 69 | 5.3 | 41.1 | 46.4 | 6.4 | 23.1 | 29.5 | 24.1 |
| | 81 | 5.9 | 37.2 | 43.1 | 4.3 | 19.6 | 23.9 | 33.0 |
| End of heat | 1073 | 6.4 | 48.1 | 54.5 | 3.3 | 8.4 | 11.7 | 33.8 |
| | 1318 | 16.1 | 25.9 | 42.0 | 1.8 | 3.9 | 5.7 | 52.3 |
| | 1322 | 7.3 | 43.1 | 50.5 | 2.0 | 4.1 | 6.1 | 43.4 |
| | 1503 | 16.7 | 20.2 | 36.9 | 2.6 | 8.8 | 11.4 | 51.8 |
| | 1512 | 15.7 | 33.4 | 49.1 | 0.8 | 3.3 | 4.1 | 46.9 |
| Fourth day | 1306 | 12.7 | 36.8 | 49.5 | 1.8 | 8.2 | 10.0 | 40.5 |
| | 1327 | 11.0 | 37.5 | 48.6 | 3.5 | 6.2 | 9.7 | 41.7 |
| | 1333 | 5.2 | 46.1 | 51.3 | 4.8 | 7.0 | 11.8 | 36.9 |
| | 1504 | 11.4 | 49.6 | 61.0 | 1.7 | 3.1 | 4.7 | 34.3 |
| | 1524 | 26.0 | 22.6 | 48.6 | 1.8 | 4.1 | 5.9 | 45.5 |
| Sixth day | R30 | 7.9 | 35.0 | 42.8 | 5.6 | 7.4 | 13.1 | 44.1 |
| | R52 | 9.2 | 37.8 | 46.9 | 6.8 | 7.3 | 14.1 | 38.0 |
| | 83 | 11.5 | 33.5 | 44.9 | 5.3 | 7.1 | 12.4 | 42.7 |
| Eighth day | 1133 | 20.2 | 30.5 | 50.7 | 3.6 | 7.0 | 10.5 | 38.7 |
| | 1180 | 26.8 | 27.6 | 54.4 | 2.9 | 4.9 | 7.7 | 37.9 |
| | 1341 | 12.1 | 33.2 | 45.3 | 2.0 | 4.5 | 6.5 | 48.2 |
| | 1389 | 15.9 | 21.2 | 37.0 | 6.5 | 17.7 | 24.2 | 38.7 |
| | 1393 | 7.3 | 40.0 | 47.3 | 4.3 | 6.9 | 11.2 | 41.5 |
| | 1394 | 26.6 | 17.5 | 44.1 | 5.3 | 18.0 | 23.3 | 32.7 |
| | 1515 | 11.7 | 31.7 | 43.4 | 2.1 | 4.5 | 6.6 | 50.0 |
| | 1859 | 12.9 | 28.3 | 41.2 | 2.4 | 3.4 | 5.8 | 53.1 |
| Tenth day | 84 | 9.7 | 32.6 | 42.3 | 6.7 | 8.8 | 15.5 | 42.2 |
| | R86 | 5.5 | 40.9 | 46.5 | 6.0 | 7.2 | 13.2 | 40.3 |
| | R91 | 7.8 | 34.3 | 42.1 | 4.3 | 6.1 | 10.4 | 47.6 |
| Twelfth day | 1120 | 8.5 | 51.1 | 59.7 | 2.3 | 3.3 | 5.6 | 34.8 |
| | 1315 | 13.7 | 29.4 | 43.1 | 1.7 | 4.7 | 6.4 | 50.5 |
| | 1317 | 16.4 | 37.0 | 53.4 | 3.0 | 4.8 | 7.8 | 38.8 |
| | 1502 | 16.5 | 30.1 | 46.7 | 2.4 | 5.1 | 7.5 | 45.8 |
| | 1522 | 13.9 | 36.8 | 50.7 | 2.4 | 5.4 | 7.7 | 41.6 |
| Fifteenth day | 912 | 6.2 | 37.7 | 44.0 | 2.3 | 8.7 | 11.0 | 45.0 |
| | 1055 | 13.2 | 36.5 | 49.8 | 3.3 | 6.5 | 9.8 | 40.4 |
| | 1325 | 8.0 | 40.7 | 48.6 | 1.8 | 3.8 | 5.7 | 45.7 |
| | 1330 | 5.4 | 38.9 | 44.3 | 3.0 | 6.5 | 9.4 | 46.3 |
| | 1506 | 24.4 | 22.2 | 46.6 | 3.2 | 8.0 | 11.2 | 42.2 |



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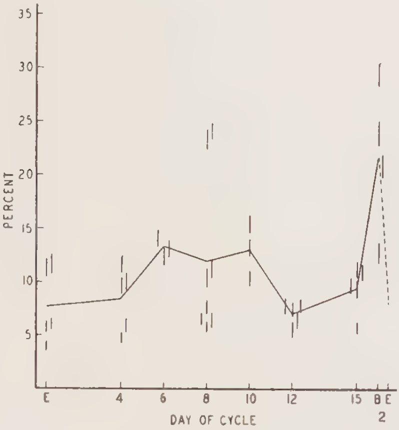

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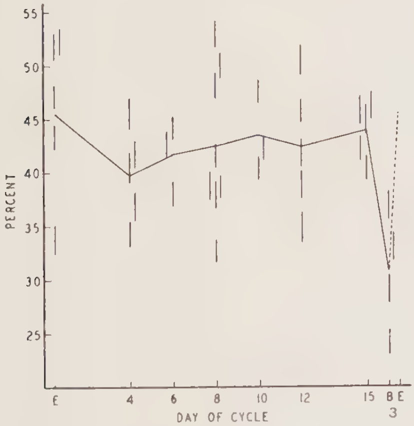
Fig. 1 Percentage of acidophiles in the anterior hypophysis of the female guinea pig during the reproductive cycle. B, beginning of heat; E, end of heat.

Fig. 2 Percentage of basophiles in the anterior hypophysis of the female guinea pig during the reproductive cycle. B, beginning of heat; E, end of heat.



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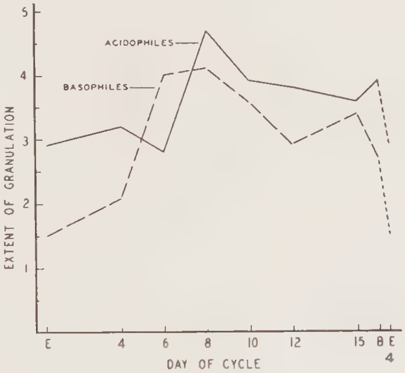

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Fig. 3 Percentage of chromophobes in the anterior hypophysis of the female guinea pig during the reproductive cycle. B, beginning of heat; E, end of heat.

Fig. 4 Extent of granulation in the acidophiles and basophiles of the female guinea pig anterior hypophysis during the reproductive cycle. Each point on the curves represents an estimate of granulation arrived at by microscopical examination. The extent of granulation is designated by arbitrary values. B, beginning of heat; E, end of heat.

reproductive cycle. The curves are presented only to show roughly the changes that occur during the cycle.

*The extent of granulation, the Golgi apparatus and the mitochondria during the reproductive cycle.* Since numerical changes in cell types could not be correlated with the reproductive cycle, an effort was made to determine if changes in any of the cellular contents are related to the cycle. An increase in granulation in both types of cells during the first half of the cycle is indicated (fig. 4). After the eighth day there is a decrease in granulation with a marked drop from the fifteenth day to the end of heat, especially in the basophiles.

Closely related to the observations on granulation were the results from a study of the Golgi apparatus and mitochondria. At the beginning of heat the Golgi apparatus is small and compact, and it is apparent in relatively few cells (fig. 5). By the fourth day it has increased slightly in size in many cells, and between the fourth and eighth days there is a marked hypertrophy and it can be seen in even more cells. From the eighth to the fifteenth day there is a tendency for the Golgi apparatus to become broken up and less visible. In the twelfth and fifteenth day groups a relatively large number of basophiles are found in which finely divided osmiophilic granules are scattered throughout the cytoplasm. It could not be determined if these granules are the result of the fragmentation of the Golgi apparatus or what their significance might be, but the fact that they were seen only in material collected on the twelfth and fifteenth days is suggestive of this possibility.

The mitochondria show a change in distribution during the cycle (fig. 5). From the beginning of heat to the fourth day they are relatively few in number and tend to be concentrated in the Golgi zone in most cells. From the fourth to the eighth day they show a slight tendency to become scattered and seem to increase in number. From the eighth to the fifteenth day they become much more scattered and apparently continue to increase in number. In some cases on the fifteenth day they are more or less concentrated at the periphery of the cell.

The concentration is also observed in the oestrous group and probably persists from the end of one cycle to the beginning of the next. The increase in number of the mitochondria during the latter part of the cycle may be only apparent because degranulation of the cell renders them more conspicuous.

These changes in the Golgi apparatus and mitochondria are more distinct in the basophiles than in the acidophiles. The light basophiles, especially, show the hypertrophy of the Golgi

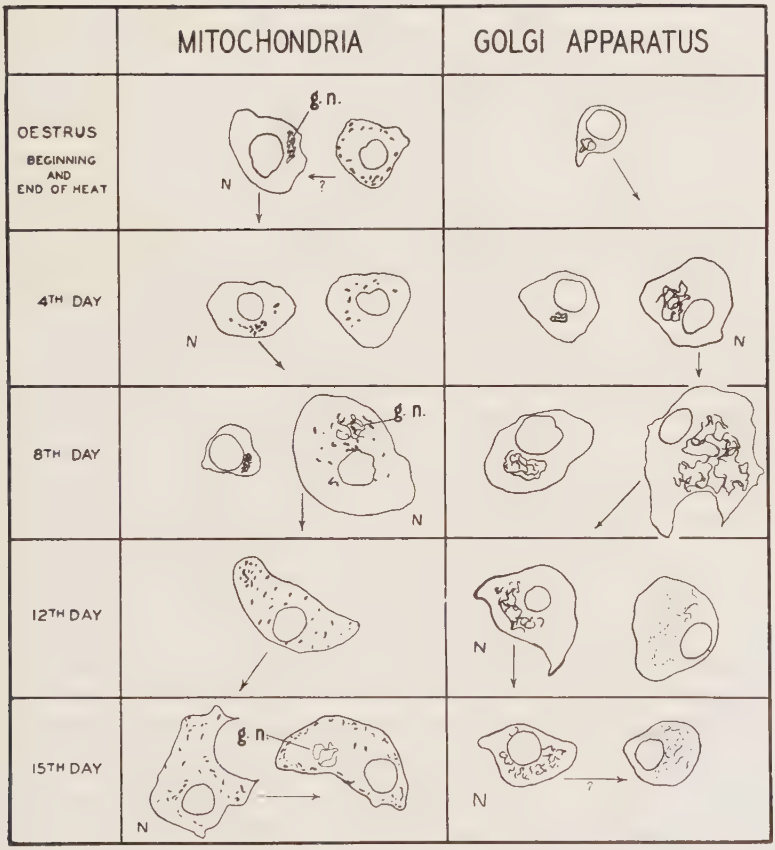

Fig. 5 Camera lucida drawings of basophiles in the anterior hypophysis of the female guinea pig illustrating changes which occur in the distribution of the mitochondria and the appearance of the Golgi apparatus during the reproductive cycle. Where two cells are shown, N denotes the type which is more numerous. g.n., Golgi apparatus. The arrows indicate the possible sequence of changes. × 850.

apparatus during the first half of the cycle, and the increase and scattering of the mitochondria during the second half, indicating that these cells which ordinarily might be thought of as physiologically identical, really represent two phases of the secretory cycle, one in which granules are being elaborated, and another in which active secretion and degranulation are taking place. Many of the acidophiles are packed so full of secretory granules that the structure of the Golgi apparatus is obscured and the mitochondria made indistinguishable. However, the structures could be seen in enough acidophiles to indicate that the changes parallel those in the basophiles rather closely.

These changes in granulation, Golgi apparatus and mitochondria suggest that during the first half of the cycle the secretory granules are being elaborated in association with the Golgi apparatus and the mitochondria, and that during the second half the granules are dissolved and discharged. The possibility will be considered more fully in the discussion of the subject given elsewhere.

*The relative percentage of cell types, the extent of granulation, the Golgi apparatus and mitochondria: their relation to the ovarian condition.* In a study of the ovarian condition and sexual behavior in the female guinea pig (Young, Dempsey, Myers and Hagquist, '38) the ovaries from the thirty-eight animals used in this investigation were examined for general follicular development, the number of ruptured follicles and the extent of rete cyst formation. Practically all the extremes of conditions that were found during the microscopic examination of 207 pairs of serially sectioned ovaries are represented in the group of thirty-eight. Consequently, comparison of the hypophyseal picture with the ovarian condition was possible, but no relationship was found. However, it is realized that although the structure of these ovaries varied greatly, all were functioning normally, and if the hypophyseal condition were compared with that in animals in which the ovaries were functioning abnormally, an entirely different situation might be encountered.

## DISCUSSION

When the results described above are compared with those obtained by the other investigators who have used the guinea pig, several differences are found. According to Chadwick ('36) the acidophiles reach their peak in early oestrus, are less numerous during the lutein phase and increase again during the follicular phase. The basophiles are high in relative percentage during prooestrum, decrease during oestrus and the lutein phase, and begin to increase during the early follicular phase. Therefore in the case of each cell type, only a single peak is shown during each reproductive cycle. With respect to granulation he reports degranulation in the acidophiles late in oestrus. The basophiles undergo degranulation early in oestrus and increase to a maximum granulation during the follicular phase. However, in considering granulation, the activity of the Golgi apparatus or the mitochondria in relation to the secretory process is not mentioned. In contrast to these observations, it was found in the present work that marked variations in percentage of cell types occur more than once during the cycle, and within each group the variations are so great that the average is not statistically significant (figs. 1 to 3). With respect to granulation, the maximum occurs in both cell types about the middle of the cycle, and therefore, toward the end of the lutein phase, rather than during prooestrum.

Kirkman's work indicates that the relative percentage of acidophiles is lower during oestrus and dioestrum. Therefore, according to him, two peaks occur. The basophiles are reported as being lowest in relative percentage at oestrus and highest during dioestrum. With respect to granulation the implication is that the acidophiles are most granular during prooestrum and degranulated during oestrus. The basophiles show extreme degranulation during oestrus and metoestrum and increase in granulation during dioestrum. These results also differ somewhat from those given here. Kirkman considers changes in the percentage of cell types significant, and that the acidophiles are most granular during prooestrum rather than during the middle of the cycle. In addition, no mention is made of the changes which occur in granulation, the Golgi apparatus and the mitochondria during the middle of the cycle.

The differences between Chadwick's results and ours may be due to the use of different fixation and staining techniques and different methods of identifying the time of heat. When an attempt is made to account for the lack of correspondence between Kirkman's results and ours, differences in cytological techniques can be eliminated because they were the same. Judgment in the identification of cell types can also be eliminated since our count of the cell types on thirteen of Kirkman's slides differed from his by only 0.3%. Therefore a possible explanation in this case, too, is that he was compelled to depend on the vaginal condition rather than the copulatory response for the determination of heat.

The extent to which our interpretation of what is happening in the cells of the hypophysis as based on changes observed in granulation, the Golgi apparatus and mitochondria, is felt to be amply justified. Numerous studies have indicated the close relationship between these structures and the secretory activity of the cell (see Wilson, '34, for the older literature). With respect to the hypophysis, Severinghaus ('33, '36, '37) points out that in general, hypertrophy of the Golgi apparatus and the presence of numerous mitochondria indicate an actively secreting cell. Gatz ('37) also associates the Golgi apparatus in the basophiles of the rat hypophysis with secretory activity. Kirkman ('37) notes that in the guinea pig the first sign of secretory activity in the basophiles is an increase in size of the mitochondria and their concentration in the Golgi zone. Following this initial activity the mitochondria become smaller and scattered, and the Golgi apparatus hypertrophies. As the cells become degranulated the Golgi apparatus diminishes in size and the mitochondria become more conspicuous. He indicates a similar cycle for the acidophile.

Hirsch ('31 a) finds that in cells of the pancreas basal granules appear first in connection with the mitochondria and that they ripen in the region of the Golgi apparatus. In a subsequent article (Hirsch, '31 b) he states that in living pancreas cells treated by x-rays the mitochondria are destroyed and are reformed during the following 2 hours as a surface layer in the cell. Only after this reformation does migration toward the Golgi zone take place. Furthermore, he believes that many secretory cells have a 'polyphasic system of secretion' in which alternate 'restitution and expulsion' of the product can take place an indefinite number of times, restitution being, in the main, a function of the mitochondria and the Golgi apparatus. Since the observations on the guinea pig suggest that during each reproductive cycle the mitochondria go through phases of concentration in the Golgi zone followed by scattering and subsequent concentration at the periphery of the cells (fig. 5), it is felt that a process comparable to that in the pancreas may be occurring in the cells of the hypophysis.

When this investigation was suggested it was thought that some cellular change in the hypophysis might be correlated with the sudden appearance of heat. The marked decrease in granulation noted as occurring about the fifteenth day may be such a change. However, the decrease seems to be simply a continuation of a process that begins about the middle of the cycle. This need not be surprising. The injection of the luteinizing hormone into normal animals as early as the twelfth day or the injection of progesterone on the thirteenth or fourteenth day of the cycle will induce heat (Dempsey, Hertz and Young, '36), indicating that the animal is conditioned several days before heat normally occurs. Consequently it may be that factors which lead ultimately to the production of heat begin to operate as early as the middle of the cycle, and when the final stimulus is received several days later, heat begins.

All that is said above presupposes that the gonadotropic activity of the hypophysis is accompanied by visible changes within the cells. The fact that evidence of a cyclic activity has been found supports this hypothesis. However, it is not surprising that signs of this activity are difficult to detect. The anterior hypophysis possesses functions besides those that are gonadotropic, and it may well be that changes related to gonadotropic activity are masked by changes associated with the other functions.

In a recent study of the hypophysis of the rabbit, cat, macaque monkey and guinea pig two types of acidophiles have been described (Dawson, '38). One stains with orange G and the other, which is the predominant type in the guinea pig, has an affinity for azocarmine. In the rabbit the carmine cells increase in number within 45 minutes after mating, dominate the cytological picture for 1½ to 4 hours, and then decrease and become inconspicuous (Friedgood and Dawson, '37). In the cat the carmine cells are present during sexual activity, but not in the anoestrous female (Dawson and Friedgood, '38). Since the fixation and staining techniques used in these investigations are not the same as ours, it is not known if the two types of acidophiles described above are comparable to those mentioned by Dawson. However, a supplementary study should enable one to relate the cells described by Dawson to those described in this article.

## SUMMARY AND CONCLUSIONS

The hypophyses of thirty-eight adult female guinea pigs were removed at eight different stages during the reproductive cycle and studied cytologically. The beginning of heat which was used as a point of reference was determined by the copulatory response. At each stage the relative percentage of cell types, the extent of granulation, changes in the Golgi apparatus and the distribution of mitochondria were determined. The hypophyseal condition was also referred to the general follicular development, the number of ruptured follicles and the extent of rete cyst formation in the ovaries.

1. Two kinds of acidophiles were identified. One has a deeply staining, homogeneous appearing cytoplasm and usually a small, dense, basophilic nucleus. The other is generally

larger, contains a granular cytoplasm, stains less intensely and has a larger and more or less vesicular, basophilic nucleus. They are thought to represent two phases of the acidophile. Light and dark basophiles comparable to those described by Kirkman ('37) were found.

2. Variations in the relative percentage of cell types during the reproductive cycle were not statistically significant. Changes in the extent of granulation, the condition of the Golgi apparatus and the distribution of mitochondria are considered indicators of activity within the cells of the hypophysis.

3. In both acidophiles and basophiles the extent of granulation increases during the first half of the cycle and decreases during the second half. The Golgi apparatus hypertrophies during the first half of the cycle and becomes fragmented in most of the cells during the second half. The mitochondria tend to concentrate in the Golgi zone early in the cycle, and after the eighth day become increasingly scattered. During prooestrum and oestrus the mitochondria in many cells are concentrated at the periphery.

4. These changes are believed to be related to processes which lead ultimately to the production of heat. They suggest, in addition, that a secretory activity associated with heat begins about the middle of the cycle rather than toward the end.

5. No relationship was found between the hypophyseal condition on the one hand, and the extent of general follicular development, the number of ruptured follicles and the extent of rete cyst formation in the ovaries on the other.

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# SPERM FORMATION AND GROWTH OF ACCESSORY REPRODUCTIVE ORGANS IN HYPOPHYSECTOMIZED GROUND SQUIRRELS IN RESPONSE TO SUBSTANCES FROM BLOOD AND HUMAN URINE [1]

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ONE TEXT FIGURE AND TWO PLATES (EIGHTEEN FIGURES)

## INTRODUCTION

Gonadotropic materials known to cause the testis of the ground squirrel (Citellus tridecemlineatus) to produce spermatozoa and secrete testis hormone are: pituitary substance, Antuitrin-S and blood serum from pregnant mares (Wells, '35 a, '37; Wells and Moore, '36; Baker and Johnson, '36). Anterior hypophysis implants in totally hypophysectomized ground squirrels have resulted in sperm production (Wells and Gomez, '37). It was deemed desirable to see if gonadotropic materials other than pituitary substance would affect the reproductive organs in hypophysectomized males of this species. The present paper reports the response of reproductive organs in hypophysectomized male ground squirrels to administration of the following substances: 1) blood serum from pregnant mares, 2) an extract of urine from pregnant women (Antuitrin-S) and 3) an extract of human male urine (Prospermin). Most of our results have been abstracted elsewhere (Wells and Overholser, '38).

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## METHODS

Reproductive organs of eighty-six male ground squirrels (Citellus tridecemlineatus) were studied grossly and microscopically in this investigation. Fifty-four of the males were hypophysectomized and they survived the operation for periods varying from 8 days to 6 months.

Hypophysectomy was accomplished by an oral approach, using both Nembutal and ether to induce surgical anesthesia. The details of hypophysectomy technique, post-operative care, periodic examination of reproductive organs by manual palpation and observation, diet of the animals and autopsy routine were identical with those published elsewhere (Wells and Gomez, '37), and for this reason they are not repeated in this paper.

Hypophysectomized animals tolerated injections poorly, especially during the first few days of treatment. Ignorance of this fact in the earlier experiments resulted in many deaths within the first week of injection. Such animals exhibited local cutaneous swelling at injection sites and also symptoms resembling those of hypoglycemia. Greater toleration was secured in the later experiments by giving small doses for the first few days. Tolerance to injection was successfully established in most cases by giving 25% of the desired dose (no more than 0.15 cc. of aqueous substance) once daily on the first and second days, 50% on the third day, 75% on the fourth and, finally, the desired dose twice daily.

In all experiments an injection period of 25 to 30 days was planned. Sixteen treated animals tolerated injections so poorly that it was necessary to terminate the treatment at an earlier date than that which had been scheduled. Thirteen of these died and autopsy was performed as soon as death was discovered; the interval between death and autopsy varied from a few minutes to a maximum of 10 hours. The other three were killed when it appeared definite that they had no chance of survival.

At autopsy the utmost care was taken in preserving possible pituitary remains. The brain was cautiously removed without destruction of the dorsal dural wall of the pituitary. In each hypophysectomized animal, the sphenoid bone was removed without disturbing the sella turcica and fixed in Bouin's fluid. This entire block of tissue was decalcified, sectioned serially at 10 μ, stained with hematoxylin and eosin and examined microscopically for fragments of pituitary tissue.

Most of the hypophysectomized animals were found to have fragments of pars intermedia and pars tuberalis, and lack pars nervosa with the exception of the stalk which almost invariably remained. Some animals showed no microscopically detectable trace of anterior lobe tissue, and such animals are referred to in this paper as 'completely hypophysectomized,' while others exhibited small fragments.

In the cases that were incompletely hypophysectomized, the amount of anterior lobe tissue remaining was calculated in percentage of the entire anterior lobe by the following method. Every third section of anterior lobe fragments was marked on the slide with an ink spot. The marked sections were then projected by an Edinger projection apparatus at a magnification of 20 and the outline of the sections drawn. The total area of these drawings was then determined in square centimeters by means of a planimeter. A complete anterior lobe from a normal animal was sectioned and measured by the same method. Actual percentages of remaining tissue in the experimental animals could thus be calculated. Figure 2 shows a section through a normal intact hypophysis. Figures 3 and 4 show sections through the sella turcica from experimental animals in which the percentages of anterior lobe fragments were 4.6 and 5.4% respectively. Figure 5 is a similar section from a series in which no anterior lobe fragments were found.

## RESULTS

### 1. Normal males

Twenty-two normal males were killed during the August to November period. Each had non-functional accessory reproductive organs and no spermatozoa (tables 1 and 2). The testis was invariably abdominal in position and small.

The above results are in harmony with those previously reported (Johnson, Foster and Coco, '33; Moore, Simmons, Wells, Zalesky and Nelson, '34; Wells, '35 a; Wells and Moore, '36). During the past 7 years, only six males exhibiting spermatozoa within the aspermatic period shown in figure 1 have been discovered by one of us (L.J.W.). These six exceptional animals were killed near the extreme ends of the aspermatic interval.

### 2. Hypophysectomized controls

Nine immature males were hypophysectomized during the aspermatic period of the year and autopsied after intervals of 44 to 93 days. Autopsied testes were small and in an abdominal position, and accessory reproductive organs were similar to those of normal controls. There were great variations in testis weight; however, in most cases, the testis weighed less than that of unoperated controls (tables 1 and 2) and showed less advance in spermatogenesis (figs. 10 and 12). Spermatozoa were not found in any case.

No consistent differences between the reproductive organs of completely hypophysectomized controls and those of partially hypophysectomized controls were noted.

### 3. Injections

*Prospermin* (extract of human male urine) was administered to twenty-two hypophysectomized males for periods of 6 to 31 days. In most cases it was dissolved in distilled water and injected subcutaneously twice daily in doses of 1.0 to 10.0 mg.

Fourteen of the animals proved to be completely hypophysectomized, eight immature and six adult. Each of five immature males injected for periods of 19 to 31 days showed large scrotal testes with spermatozoa (table 1, fig. 14), and accessory organs similar to those of normal breeding adults (fig. 15). The average weight of accessory organs was approximately 27 (bulbar gland) to 63 (seminal vesicles) times greater than that for untreated controls, and the height of the prostate epithelium was about 2 to 3 times greater. Three other immature males injected for periods of 10 to 18 days exhibited no spermatozoa. Each of six adults possessed scrotal testes with sperm following an injection period of 20 to 31 days. One of these adults was killed during the normal breeding season; however, at laparotomy preceding injection one testis was removed and found to be small, abdominal and aspermatic.

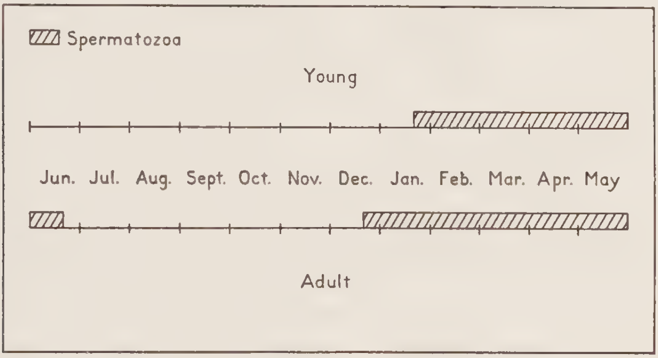

Fig. 1 Occurrence of spermatozoa in 511 normal males autopsied (232 cases) or castrated (278 cases) during the period 1931 to 1938.

Eight males had anterior lobe fragments. Six of these had spermatozoa at autopsy, while two injected for intervals of 6 to 10 days had none. The presence of spermatozoa in one animal killed on January 29th is not considered significant, due to the fact that it had a large fragment of the anterior hypophysis and was killed during the season of sperm expectancy in unoperated controls.

TABLE 1[1]

*Effects of Prospermin on testis and accessory glands of hypophysectomized males*

| AGE | NO. | DAYS HYPOPHYSECTOMIZED | TREATMENT | | MONTH KILLED | BODY WEIGHT | PER CENT HYPOPHYSIS TISSUE | TESTIS | | ACCESSORY ORGANS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Milligrams daily | Days | | | | Weight | Sperm [5] | Prostate gland Weight | Prostate gland Epithelium | Bulbar gland weight | Cowper's glands weight | Seminal vesicles weight |
| | | | | | | *gm.* | | *gm.* | | *gm.* | *µ* | *gm.* | *gm.* | *gm.* |
| Immature | 4 | 31–40 | 5.0 | 19–26 | Nov. | 119 | 0.0 | 0.6358 | + | 0.1813 | 21.0 | 0.7233 | 0.3065 | 0.7371 |
| | 2 | 38 | 5.0 | 26 | Nov. | 160 | 50.0 | 0.7055 | + | 0.2243 | 23.5 | 0.9692 | 0.3378 | 1.0397 |
| | 3 [4] | 30–37 | 1.0–2.0 | 10–18 | Jan. | 107 | 0.0 | 0.3491 | 0 | 0.0632 | 16.1 | 0.2067 | 0.0894 | 0.1566 |
| | 1 [4] | 92 | 3.0 | 31 | Jan. | 145 | 0.0 | 0.7546 | + | 0.2017 | 24.9 | | | 0.8806 |
| | 2 [4] | 30–67 | 3.0–10.0 | 6–10 | Jan. | 159 | 8.7 | 0.4040 | 0 | 0.0456 | 20.4 | 0.1684 | 0.0728 | 0.1172 |
| | 1 [2,4] | 92 | 5.0 | 31 | Jan. | 190 | 45.0 | 0.8550 | + | 0.2841 | 26.6 | | | 2.0051 |
| | 4 | 49–93 | None | | Oct.–Jan. | 142 | 0.0 | 0.1858 | 0 | 0.0033 | 8.0 | 0.0258 | 0.0054 | 0.0094 |
| | 5 | 44–63 | None | | Aug.–Jan. | 135 | 19.5 | 0.1343 | 0 | 0.0036 | 8.5 | 0.0252 | 0.0059 | 0.0117 |
| | 4 | Normal | None | | Nov. | 178 | Normal | 0.2522 | 0 | 0.0042 | 11.0 | 0.0266 | 0.0060 | 0.0090 |
| Adult | 5 | 179–185 | 5.0 | 20–26 | Nov. | 145 | 0.0 | 0.7346 | + | 0.1904 | 20.5 | 0.7506 | 0.4001 | 0.9326 |
| | 3 | 38–185 | 5.0 | 26 | Nov. | 145 | 5.9 | 0.8654 | + | 0.2372 | 21.7 | 0.7779 | 0.4624 | 1.0718 |
| | 1 [3] | 140 | 5.0 | 31 | May | 132 | 0.0 | 0.6626 | + | 0.2591 | | 0.4802 | 0.3508 | 1.3184 |
| | 2 | Normal | None | | Nov. | 236 | Normal | 0.3033 | 0 | 0.0135 | 4.3 | 0.1209 | 0.0271 | 0.0375 |

[1] Data expressed as averages for each group.

[2] Autopsy date and presence of pituitary fragment make sperm presence not significant.

[3] One testis surgically removed on April 19th before injection. It weighed 0.1695 gm. and contained no spermatozoa.

[4] Prospermin suspended in olive oil.

[5] Sixteen of seventeen spermatic males showed spermatozoa in fresh epididymis smears, and eight exhibited sperm motility.

## TABLE 2[1]

*Effects of mare serum and Antuitrin-S on testis and accessory glands of hypophysectomized males*

| AGE | NO. | DAYS HYPO-PHYSEC-TOMIZED | TREATMENT | | | MONTH KILLED | BODY WEIGHT | PER CENT HYPOPHY-SIS TISSUE | TESTIS | | ACCESSORY ORGANS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Prostate gland | | | | |
| | | | Sub-stance | R.U. daily | Days | | | | Weight | Sperm[5] | Weight | Epi-thelium | Bulbar gland weight | Cowper's glands weight | Seminal vesicles weight |
| | | | | | | | gm. | | gm. | | gm. | μ | gm. | gm. | gm. |
| Immature | 4 | 16–19 | M.S. | 40 | 9–12 | Aug. | 117 | 0.0 | 0.1234 | 0 | 0.1257 | 14.5 | 0.1421 | 0.0695 | 0.1470 |
| | 2 | 51–55 | M.S. | 40 | 29–33 | Oct. | 112 | 0.0 | 0.5119 | + | 0.1394 | 19.7 | 0.2886 | 0.2226 | 0.5032 |
| | 5 | 56–59 | M.S. | 20–40 | 30–37 | Sept.–Oct. | 120 | 13.7 | 0.4232 | + | 0.1244 | 19.6 | 0.2584 | 0.2063 | 0.5257 |
| | 4 | 17–30 | Ant. | 100–200 | 10–16 | Aug. | 105 | 0.0 | 0.1197 | 0 | 0.0419 | 15.5 | 0.1230 | 0.0687 | 0.1370 |
| | 3 | 53–88 | Ant. | 50–75 | 31–42 | Sept.–Oct. | 105 | 0.0 | 0.3950 | + | 0.1990 | 18.6 | 0.4128 | 0.3483 | 0.9027 |
| | 1 | 63 | Ant. | 75 | 37 | Oct. | 115 | 3.9 | 0.3850 | + | 0.1860 | 24.0 | 0.4950 | 0.3226 | 0.6200 |
| | 2 | 44 | None | | | Aug. | 121 | 9.0 | 0.0956 | 0 | 0.0038 | 10.7 | 0.0179 | 0.0061 | 0.0097 |
| | 2 | 58 | None | | | Oct. | 133 | 0.0 | 0.1625 | 0 | 0.0034 | 8.6 | 0.0216 | 0.0046 | 0.0094 |
| | 6 | Normal | None | | | Aug. | 134 | Normal | 0.0448 | 0 | 0.0038 | 10.6 | 0.0224 | 0.0068 | 0.0101 |
| | 2 | Normal | None | | | Oct. | 174 | Normal | 0.1905 | 0 | 0.0048 | 11.1 | 0.0240 | 0.0066 | 0.0112 |
| Adult | 1[2] | 17 | Ant. | 50 | 13 | Aug. | 125 | 0.0 | 0.2242 | 0 | 0.1170 | | 0.4136 | 0.4426 | 0.3994 |
| | 1[3] | 8 | Ant. | 100 | 6 | Aug. | 190 | 66.0 | 0.3818 | 0 | 0.0444 | 10.7 | 0.1545 | 0.1185 | 0.1275 |
| | 1 | 19 | M.S. | 40 | 16 | Aug. | 170 | 0.0 | 0.4100 | 0 | 0.2992 | 21.5 | 0.6006 | 0.8181 | 1.1800 |
| | 1[4] | 139 | M.S. | 25 | 30 | May | 140 | 0.0 | 0.4696 | + | 0.2825 | | 1.0757 | 0.4579 | 1.5337 |
| | 8 | Normal | None | | | Aug. | 223 | Normal | 0.1443 | 0 | 0.0193 | 5.8 | 0.1606 | 0.0396 | 0.0414 |

[1] Data expressed as averages for each group.

[2] At laparatomy on August 8th before injection, testis size was 9.0 × 4.0 mm. Testis at autopsy 12.0 × 6.0 mm.

[3] At laparotomy on July 31st before injection, testis size was 12.0 × 6.0 mm. Autopsy testis 14.0 × 7.0 mm.

[4] One testis surgically removed on April 19th before injection. Testis weighed 0.1959 gm. and contained no spermatozoa.

[5] Two of four Antuitrin-S spermatic males had motile sperm in epididymis. Seven of eight mare serum males with testicular sperm also had spermatozoa in the epididymis; sperm motility observed in four of these.

Some of the average weights in animal groups of table 1 are somewhat greater for injected animals with hypophysis fragments than are the average weights for treated males with no detectable anterior lobe tissue. Comparison of protocols for individual animals showed that this difference in weight was related to a variation in the length of the injection period and not to the presence or absence of an anterior lobe fragment.

Ten non-hypophysectomized immature males were castrated bilaterally for 71 days. Nine of these were injected twice daily for 30 days with doses ranging from 0.5 to 10.0 mg. suspended in olive oil. The nine injected animals and one untreated castrate were killed on the same day. The following data represent averages for the nine injected castrates, and these data are compared respectively with those in the uninjected castrate: body weight, 206 and 210 gm.; prostate gland, 0.0036 and 0.0037 gm.; height of prostate epithelium, 7.3 and 8.6 μ; seminal vesicles, 0.0079 and 0.0094 gm. Since no constant significant difference was found between accessory organs of injected and untreated castrates, it is concluded that prospermin in the above dose range exhibited no androgenic potency.

*Blood serum from pregnant mares* was given to eleven immature and two adult hypophysectomized males in two daily subcutaneous injections for periods of 9 to 37 days. The daily dose ranged from 20 to 40 rat units.

Six immature and two adult males were completely hypophysectomized. Two immature males following 29 to 33 days of injection (table 2, figs. 16 and 17), showed large scrotal testes with spermatozoa and functional accessory reproductive organs. Average weight of accessory organs was approximately 12 (bulbar gland) to 44 (seminal vesicles) times greater than that for untreated controls. The remaining four immature males exhibited lesser responses to shorter injection periods which varied from 9 to 12 days. These short periods failed to cause testicular descent and sperm formation, but they resulted (probably indirectly through the testis) in a great stimulation of accessory organs. One completely hypophysectomized adult had small, abdominal and aspermatic testes, one of which was removed at laparotomy; treatment for 30 days caused the remaining testis to enlarge, descend into the scrotum, and produce spermatozoa; accessory organs attained a size equal to that for normal breeding adults. In the other adult, 16 days of injection caused a stimulation of all reproductive organs but no sperm production.

Five immature males had anterior lobe fragments. Treatment for 30 to 37 days caused a stimulation of the entire reproductive tract in each case. At autopsy, testes were large, scrotal and spermatic, all accessory organs were large, and the epithelium of the prostate gland was higher than that of control animals (table 2).

*Antuitrin-S* was given subcutaneously to eight immature and two adult hypophysectomized males twice daily in doses of 25 to 100 rat units for periods of 6 to 42 days.

Seven immature animals and one adult were found to be completely hypophysectomized. Three immature males injected for 31 to 42 days had large, scrotal testes with spermatozoa (table 2, fig. 18). Average weight of their accessory organs was approximately 8 (bulbar gland) to 80 (seminal vesicles) times greater than that for untreated controls. The average height of the epithelium of their prostate glands was about two times greater than that for controls (fig. 19). Four other immature males were treated for only 10 to 16 days. Their testes were abdominal in position and aspermatic, and their accessory organs weighed about 6 to 10 times more than those of untreated males. The adult male receiving only 13 days of treatment had reproductive organs that were definitely larger than those of control adults. No spermatozoa were found in this animal.

One immature and one adult male had anterior lobe fragments. All reproductive organs of the immature male were greatly stimulated after 37 days of treatment. The testes had descended into the scrotum and produced spermatozoa. The adult was injected for only 6 days. Its reproductive organs were larger than those of uninjected adult controls. This adult did not have spermatozoa.

## DISCUSSION

Formation of spermatozoa in response to these substances in the absence of the hypophysis is significant mainly in the fact that sperm production was induced in immature as well as adult males and at a period of the year when spermatozoa are normally absent in this species. Anatomically mature spermatozoa with or without motility were found to be abundant in the epididymis in most cases. Also, it is an interesting fact that Prospermin, an extract of human male urine, was found to have gonadotropic and no androgenic properties.

Immature ground squirrels have been previously reported to undergo precocious sperm production, and adults showing seasonal aspermia have been stimulated to renewed sperm formation in response to Antuitrin-S and mare serum (Wells, '35 a, '35 b; Wells and Moore, '36; Baker and Johnson, '36). The ferret, another seasonal breeder, when subjected to an increase in the length of day and injections of pregnancy urine, produced sperm earlier in the year than untreated controls (Allanson, Rowlands and Parkes, '34). Morrell ('33) reported that precocious spermatogenesis was induced in immature rats by injections of Prospermin combined with either a growth preparation of beef pituitaries or pituitary hebin. Moore ('36) has reviewed the work of investigators who have reported upon the effects of these substances on spermatogenesis in immature mice and rats; Moore found that these materials (mare serum, Antuitrin-S and Prospermin) did not hasten spermatogenesis in immature rats to the 'sperm-head stage,' this stage proving to be a dependable and effective end point. Cole ('36) reported no sperm production in rats following pregnant mare serum injections. In adult human hypogonadism, Antuitrin-S has been found to cause the appearance of spermatozoa in condom samples of semen (Brosius and Schaffer, '33).

Smith and Leonard ('33) have shown that pregnancy urine extracts will maintain or repair spermatogenesis in hypophysectomized rats. Other urinary and pituitary substances have also been found capable of regulating spermatogenesis in hypophysectomized rats (Smith, Engle and Tyndale, '34; Evans, Pencharz and Simpson, '34; Greep and Fevold, '37). Although it seems clear from the above reports that sperm formation can be readily induced in adult rats, spermatogenesis in rats immature at the time of hypophysectomy has resulted rarely, if ever, in the formation of fully mature spermatozoa with motile capacity such as we are able to report for immature hypophysectomized ground squirrels.

It has been our experience that the length of the injection period is probably more important than the size of the dose, since 19 days was the shortest period of treatment resulting in spermatozoa formation and the substances were injected in large overdoses in most cases. We feel that the optimum length of treatment should be about 30 days.

## SUMMARY

1. Immature ground squirrels normally do not produce spermatozoa before late January, at which time they are about 7.5 months old. Normal adults have small, abdominal and aspermatic testes for a period of about 6 months between July and December.

2. Hypophysectomy was performed during months of aspermia, and surgical success was checked by a study of serial sections of the sella turcica. Volume of anterior hypophysis fragments in cases of incomplete hypophysectomy was measured by means of an Edinger projection apparatus and a planimeter.

3. Adequate treatment of hypophysectomized males with extracts of human male urine, pregnancy urine and pregnant mare serum caused the testis to enlarge, descend into the scrotum and produce spermatozoa. Accessory reproductive organs of some animals reached a size and histological state characteristic of normal breeding adults.

4. Both immature hypophysectomized males and hypophysectomized adults were stimulated to produce sperm, which in most cases were present in the epididymis and in some cases were motile.

5. Sperm production occurred equally as well in the absence of the anterior hypophysis as in animals with anterior lobe fragments.

6. The substances were deliberately administered in overdoses; therefore, our data have more qualitative than quantitative value.

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## PLATE 1

### EXPLANATION OF FIGURES

2 Section through sella turcica of normal ground squirrel showing hypophysis. N, pars nervosa (posterior lobe) surrounded by pars intermedia. D, pars distalis (anterior lobe). Pituitary stalk and pars tuberalis lie to the right of the anterior lobe. × 15.

3 Section through sella turcica of hypophysectomized ground squirrel no. 1405. Volume of the anterior lobe fragment, D, was found to be 4.6%. × 15.

4 Section through the sella turcica of hypophysectomized ground squirrel no. 1406. Volume of anterior lobe fragment, D, was 5.4%. N, pars nervosa surrounded by pars intermedia. × 15.

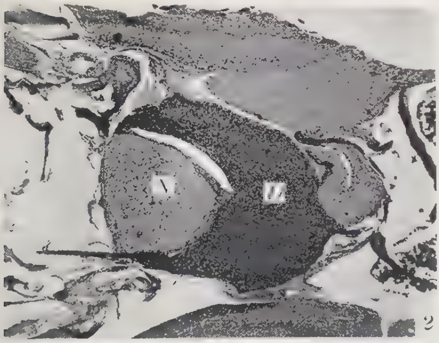
5 Section through the sella turcica of hypophysectomized ground squirrel no. 1403, which was 'completely hypophysectomized' so far as anterior lobe is concerned. N, pars nervosa surrounded by pars intermedia. × 15.

6 Section of testis from normal adult killed December 3rd. Note absence of spermatids and spermatozoa. × 200.

7 Section of prostate gland from above animal. × 200.

8 Section of testis from adult hypophysectomized male no. 1407 which received Prospermin and was killed November 28th, 184 days after hypophysectomy. This animal received 5.0 mg. Prospermin daily for 26 days. No anterior lobe tissue remained. Many motile spermatozoa found in epididymis. × 200.

9 Section of prostate gland of above animal. × 200.



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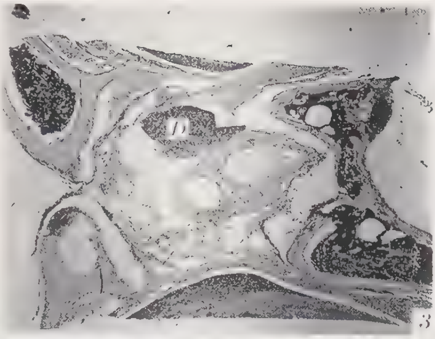

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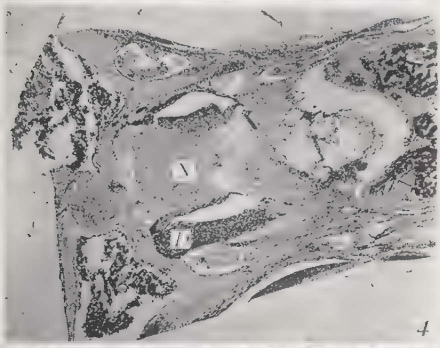

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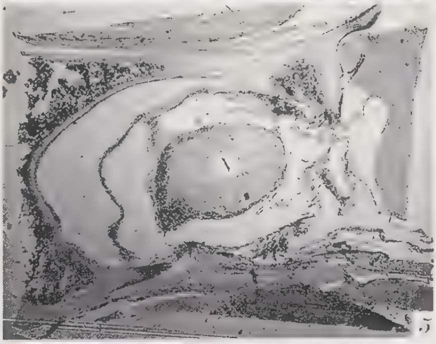

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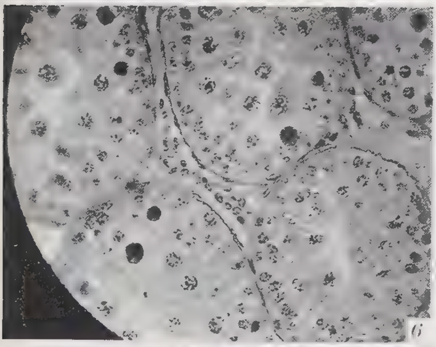
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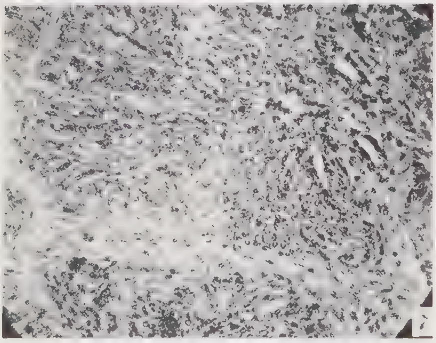
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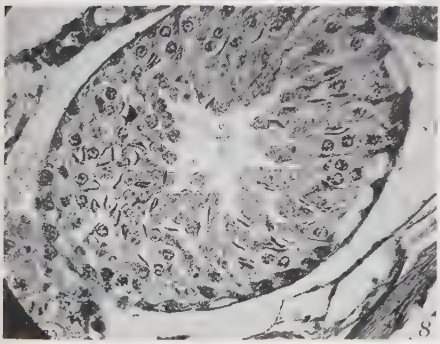
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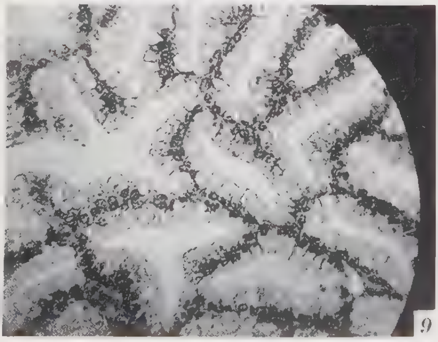
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## PLATE 2

### EXPLANATION OF FIGURES

10 Section of testis from normal immature male killed November 19th. Note absence of spermatids and spermatozoa. × 200.

11 Section of prostate gland from above animal. × 200.

12 Section of testis from uninjected hypophysectomized immature male no. 1424 which was killed on November 28th, 46 days after hypophysectomy. No spermatids or spermatozoa. Volume of anterior lobe fragment was 65%. × 200.

13 Section of prostate gland from above animal. × 200.

14 Section of testis from immature hypophysectomized male no. 1412 which received 5.0 mg. Prospermin daily for 26 days and was killed November 28th, 40 days after hypophysectomy. No anterior lobe tissue. Many spermatozoa in the testis and in the epididymis. × 200.

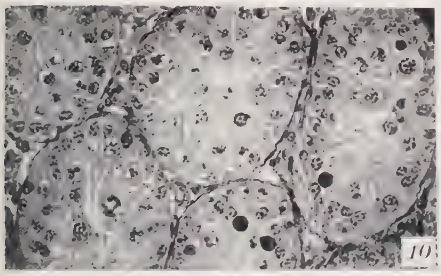
15 Section of prostate gland of above animal. × 200.

16 Section of testis from immature hypophysectomized male no. 1279 which received 40 rat units of mare serum daily for 33 days and was killed on October 27th, 55 days after hypophysectomy. No anterior lobe tissue. Many spermatozoa in testis. Many motile sperm from epididymis. × 200.

17 Section of prostate gland from above animal. × 200.

18 Section of testis from immature hypophysectomized male no. 1258 which received 75 rat units of Antuitrin-S daily for 42 days and was killed on September 24th, 70 days after hypophysectomy. No anterior lobe tissue. Many spermatozoa in testis and epididymis. Few epididymis sperm showed motility. × 200.

19 Section of prostate gland from above animal. × 200.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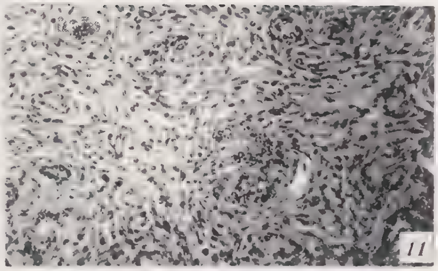
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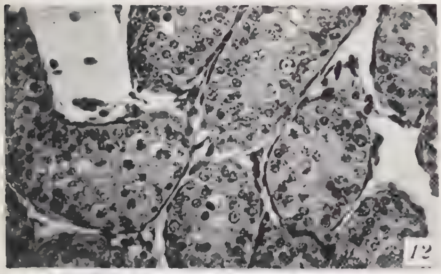
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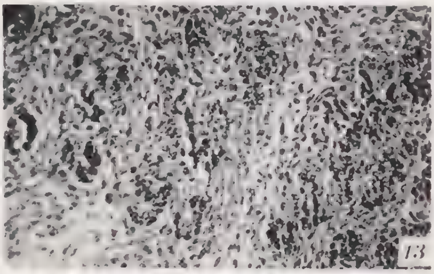
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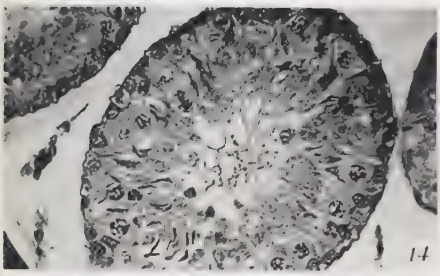
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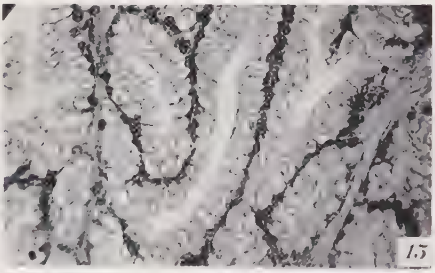
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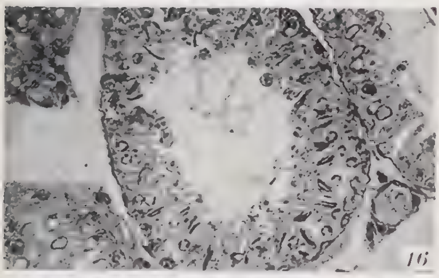
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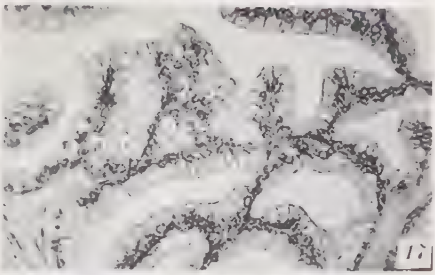
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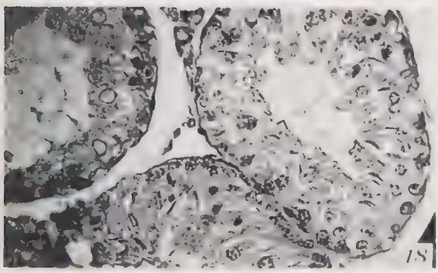
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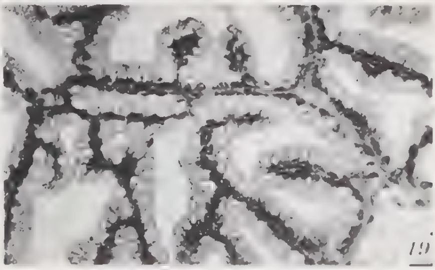
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# A STUDY OF CORTICOADRENAL CELLS

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TWO TEXT FIGURES AND TWO PLATES (TWELVE FIGURES)

## INTRODUCTION

The significance of the different kinds of cells found in the adrenal cortex, depends to some extent on whether each of the cell types have different functions and are derived independently or whether the various types represent functional states of the same cell. Evidence favoring the continuity of the cell types and the growth of the adrenal cortex from without inward has been recently summarized by Hoerr ('31). The present paper deals briefly with normal adrenal cortex histology and presents some cytologic observations bearing on the origin of new and removal of old corticoadrenal cells under experimental conditions.

## TECHNIQUE

Amphibian, rodent, carnivore and primate adrenal glands have been studied. The glands were obtained by operation or at necropsy from normal, from experimentally treated and from pathologic cases. Immature and embryonic types were also examined. They were fixed in formalin, Burke's pyridine-formalin ('33), Bouin's, Helly's, or Zenker's fluids. For certain cytological details the adrenals were removed and fixed entire in freshly prepared Allen B-15 (McClung, '33) fluid warmed to 30°C. A plug of absorbent cotton was placed in the bottom of the vials to insure even access of the fixative to the whole gland, and the original fixing fluid was replaced after a half an hour with a freshly prepared solution. Many mammalian adrenals were obtained by surgical procedure under ether anesthesia. With rapid removal of the glands and manipulation reduced to minimum, the time from clamping off the adrenal blood vessels to the immersion in fixing solution was not over 1 minute. Paraffin and celloidin sections were stained with Mallory's or Masson's triple stain, acid fuchsin and Meyer's hematoxylin and eosin. Fresh and gelatin-embedded (Zwemer, '33) frozen sections were stained with a variety of lipoid stains. The one most frequently used was Sudan IV, counterstained with hematoxylin; and the addition of Malachite green to these was found to be useful. Mounting media of various refractive indices were tried in an effort to bring out more detail and improve definition. The most satisfactory were hyrax (N, 1.81), glychrogel (N, 1.71), balsam (N, 1.53) and diaphane green (N, 1.48).

## OBSERVATIONS

The cell types found in a normal, adult, mammalian adrenal cortex will be described in the order in which they may be observed when studying the gland from the capsule toward the medulla.

The capsule is usually composed of spindle-shaped fibroblast-like cells with long processes intimately associated with typically staining connective tissue fibers. Some rounder macrophage-like cells are usually present. In certain regions of the capsule, especially at the inner border near the glomerular region and in trabeculae penetrating into the gland, are cells which differ in having shorter processes and being more rounded, with the cytoplasm more evenly distributed around the nucleus. The latter is clear, oval and contains several scattered chromatin bodies, but no definite nucleolus. Different degrees of shortening and rounding of the cell body may be readily observed (figs. 1 and 9) in a single section. With fat stains one can see that the rounder cells contain lipoid droplets.

The sub-capsular cells of the 'zona glomerulosa' are distinctly glandular. The nuclei are large and spherical with a well-defined, deeply staining nucleolus. These pyramidal or prismoid cells under normal conditions have a fair amount of cytoplasm and contain some lipoid. Their nuclei frequently show evidence of mitotic proliferation. Their frequent association in rounded or arched groups seems to be inconsistent with the conception of continuity of cell types but may be explained by the following observations. A growth arc (figs. 2 and 3) when cut longitudinally would show the glandular cell column to be arched at the top and independent of the capsule (fig. 2, E-F). The arched tops

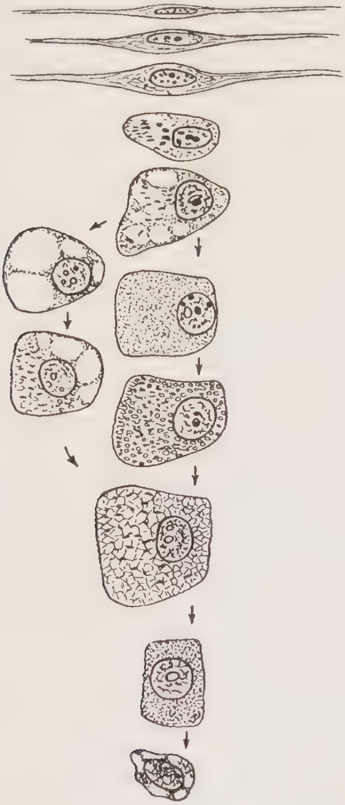

Fig. 1 Drawings of some of the stages in the life history of corticoadrenal cells.

may sometimes be cut to lie separately, giving the appearance of cell clumps which have been incorrectly called 'glomeruli' (fig. 2, C-D). Rapid cellular multiplication in experimentally activated glands seems to be responsible for gland cellular foci in the capsule. When cut obliquely or transversely these foci appear as islands of cells surrounded by connective tissue (fig. 2, A-D, fig. 5). Such clumps or closely packed small cells were shown by serial sections to be the starting point for cell columns, which extended inward in the typical manner toward the medulla, or outward to join with or become accessory adrenal tissue.

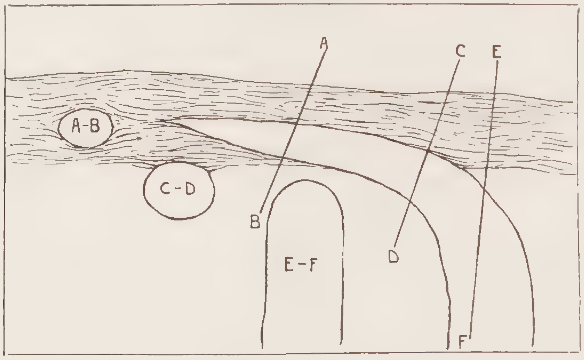

Fig. 2 Diagram of an arc of corticoadrenal cells with the results of sections through various portions of it (fig. 3). A-B, cell clump in capsule (see fig. 5). C-D, so-called glomerulus (see fig. 4). E-F, arched top of column commonly observed (see fig. 6).

When capsular material projects into the gland substance in actively-growing, young adult glands, the trabeculae do not separate cell columns, as the latter are seen radiating from the sides of the connective tissue projection (fig. 6). A transverse section of a trabeculum shows a small strip of capsule cells centrally in the cell mass, from which the fascicular columns appear to radiate. The reticularis of this small 'inverted gland' mingles with that of the gland proper or terminates at the medulliadrenal border (figs. 7 and 8).

Under normal conditions, cells below the capsule contain an increasing amount of lipoid material, evenly distributed throughout the cytoplasm in small droplets (figs. 4 and 10). In chronic toxic conditions much larger droplets may be present in these sub-capsular cells.

The cells in the outer fascicular zone stain very lightly with the usual cytoplasmic stains and appear pale and granular. Close study of properly fixed and mounted paraffin or celloidin sections showed that the granules were actually the junctures or knots of a very fine protoplasmic network (fig. 11). With gelatin-freezing technique it was seen that this network enclosed the small, uniformly distributed lipoid droplets (fig. 12). In poor paraffin preparations the network is sometimes broken giving the impression of a much larger droplet size. Large droplets may also form with prolonged use of some fat stains. This is apt to be misleading since in certain physiologic or pathologic conditions large fat droplets may actually be present.

Inward from the 'spongiocytes' the cells gradually diminish in size. This may be due to secretion loss or a condensation of their cytoplasm. Frequently this cytoplasmic difference was found to be abrupt, and a fairly definite line of demarcation occurred between the large light staining outer fascicular and the smaller inner fascicular cells (fig. 13). These inner cells stain well with the usual cytoplasmic stains and do not contain as many visible lipoid droplets.

The inner fascicular cells frequently contain small basophilic granules which stain with hematoxylin. These granules or similar ones seem to have reducing properties since they may be observed after treatment with osmium tetroxide or silver nitrate. Those reducing silver nitrate have been associated by Bourne ('33) with the presence of vitamin C.

Certain so-called dark and light cells found in paraffin sections of the reticularis of the adrenal cortex might be explained on the basis of lipoid emulsification. In the light cells the protein material which takes the cytoplasmic dye, is thinly distributed between many lipoid spheroids. On the other hand, when the fatty material is present in a few large sized globules the continuous phase is denser and gives the appearance of a darkly stained cell. The dark cells are usually seen in small groups of two or more packed between their lighter staining neighbors.

Near the medulla (fig. 14) are found pyknotic nuclei and cellular debris, which may be increased in amount after experimental procedures devised to increase the physiologic demands that have been made on the gland. Macrophages are also present in the interstitial spaces and on the endothelial walls. Fat near the medulla is for the most part in the macrophages and capillary sinusoids and not in the glandular cells of the reticularis.

Variations in capillary size are interesting in their reciprocal relation to cell size and content. The small arteries in the capsule break up at once into vessels having only endothelial lining and a few reticular fibers for support. These capillaries perforate the gland in large numbers so that each cell appears to have a capillary surface. In fixed normal tissue the vessel lumen in the region of the large outer fascicular cells is almost obliterated. Further inward, where the columns are narrower and the cells smaller, the cortical capillaries correspondingly increase in size; the endothelium being in direct contact with the gland cell surface. In the region of cell degeneration and disintegration, anastomoses between the sinusoids are most frequent, and the cell arrangement appears in sections as a network (reticular zone, fig. 14).

Mitochondria are seen in the protoplasmic halo around the nucleus of the sub-capsular cells. When the lipoid is present in many small droplets, as in the outer fascicular cells, the mitochondria appear to be increased in number so that the cytoplasm is filled with finely emulsified lipoid material with the chondriosomes at the interfaces (Hoerr, '36). The mitochondria seemed to be parallel to each other with their long axes extending from the nucleus toward the capillary surface of the cell. Such an orientation has been observed in the cells of other organs (Pollister, '32). This arrangement is somewhat disturbed if the cells are heavily loaded with large lipoid droplets.

## DISCUSSION

The sequence of corticoadrenal cell morphology was observed in the light of the following characteristics; nuclear size and appearance, cell-shape, lipoids and chondrioplasts. Special attention has been paid to the forms which are transitional between the usually recognized cell types in the adrenal cortex. As a result of studying sections prepared by a number of different techniques we feel that we can support the view that the adrenal cortex grows from without inward, and that new glandular cells are derived by a morphogenesis from indifferent cells of the capsule. It would seem that the various regions of the cortex exhibit the different morphological states occurring during the life history of the corticoadrenal cells, which might be expressed dynamically as follows.

Under normal conditions there is multiplication of the subcapsular cells by mitotic division followed by a gradual inward migration as observed by Mulon ('03), Bogomolez ('09), Graham ('16), Goormaghtigh ('22), and Hoerr ('31).

Our observations indicate that new sub-capsular cells are constantly being formed from previously undifferentiated cells in the capsule. With experimental procedures, the adult adrenal cortex may show an accelerated differentiation from these cells. The sequence of the steps of differentiation can be emphasized by experiments such as thyroid administration to a cat or by dehydration of a frog as is illustrated in figure 9. It is interesting to note in this connection that the presence of the capsule is important for successful transplantation of corticoadrenal tissue in the rat (Wyman and Walker, '29; Ingle and Higgins, '38).

Transitions in the structure of the corticoadrenal cells indicate that their growth or accretion stage takes place in the short transitional zone between the capsule and the mature fascicular cell (fig. 10). The latter (figs. 11 and 12) probably elaborates a secretion which it then discharges with more or less rapidity into the blood stream. Since the lipoid-filled cell is the form common to the cortico-adrenal gland of all the species studied, and since the vital hormone of the adrenal cortex has been associated with sterol lipoids, special attention must be given to the gradual increases and decreases of cell lipoids and the resultant lipoid distribution in the various zones. This has been expressed by previous authors as differences in the amount and distribution of corticoadrenal lipoids (Deanesly, '31). Species differences in the proportions of the various cell types should not be forgotten, as their relative proportions as compared to the normal of that species are important in estimating the physiological activity of the gland (Zwemer, '34, '36).

Loss of lipoid from the cell may be an extrusion phenomenon. Tissues fixed during active discharge of the adrenal showed lipoid droplets in the sinusoidal capillaries of the cortex, and in the larger veins traversing the medulla. Observation of the living adrenal (Singer and Zwemer, '34) revealed that after injection of toxin the cell granules swelled to large droplets which projected into the lumina of the capillaries. Similar projections of lipoid on the endothelial surfaces of corticoadrenal cells have frequently been observed in fixed tissue. It would appear that the corticoadrenal cells may in some cases secrete lipoids directly into the capillaries as visible droplets. This discharge probably continues in the inner fascicular and outer reticular regions and the cell finally degenerates in the inner reticular zone.

The inward migration of corticoadrenal tissue might be compared to the slow movement of a viscous liquid. Rapid proliferation and hypertrophy of the cells would force them inward as a slow moving mass, perforated by the longitudinal capillaries and their cross anastomoses. This does not seem so impossible when one considers that the gland has a very high lipoid and water content (Goldzieher, '29; Hoerr, '31). With the internal supporting structure consisting only of reticular fibers lying beneath the endothelium lining the vessels, the cells should be able to migrate easily.

Many investigators have confirmed the postnatal involution of the inner zone of the adrenal cortex first described by Starkel and Wegrzynoski ('10) and Thomas ('11). The regeneration of the gland from the unaffected outer zone was studied in 200 cases from the pathological files of The Sloane Hospital for Women, New York City by Zwemer, Schwartz and Parson (unpublished). The depth or extent of the organized columnar portion of the cortex from the capsule inward appeared to be dependent upon the length of postnatal survival, which is compatible with the present thesis that the adrenal cortex grows from its capsule inward toward the medulla.

## SUMMARY

1. Careful study of many adrenal glands of primates, carnivores, rodents and amphibians have been made by a variety of techniques.

2. These studies support the view that the various morphological types found in the adrenal cortex are different stages in the life history of the same cell.

3. Our present observations suggest that gland cells in the adult adrenal cortex may be derived from certain indifferent cells found in the capsule.

4. If our interpretations are correct, these capsule cells lose their long processes, differentiate into subcapsular cells, multiply, accrue lipoid, and become large outer fascicular cells. An elaborated secretion is discharged into the capillaries by these cells which gradually become smaller and finally degenerate at the medullary border.

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# PLATES

## PLATE 1

### EXPLANATION OF FIGURES

3 Monkey adrenal gland cut to show columns arching out from capsule. Paraffin section, Masson trichrome stain. × 250 (see fig. 2).

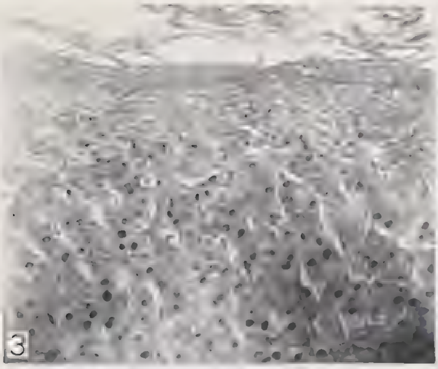
4 Human adrenal gland showing lipoid increase from capsule inward. Gelatin-frozen section, Sudan IV-hematoxylin stain. × 250 (see fig. 2, C-D).

5 Cell clump in adrenal capsule of a cat. Paraffin section, Mallory triple stain. × 500 (see fig. 2, A-B).

6 Cat adrenal gland showing cell columns arising from a trabeculum. Paraffin section, Masson trichrome stain. × 100 (see fig. 2, E-F).

7 Introverted corticoadrenal cell group of a cat. Paraffin section, Mallory's triple stain. × 50.

8 Another introverted clump of corticoadrenal cells under higher power. Paraffin section, Mallory's triple stain. × 100.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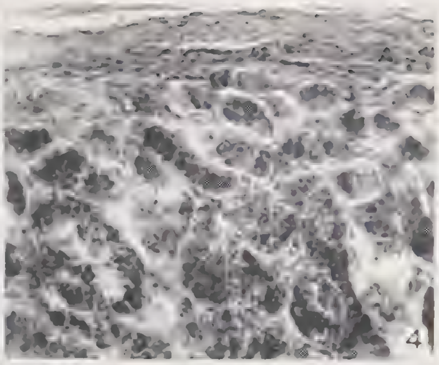
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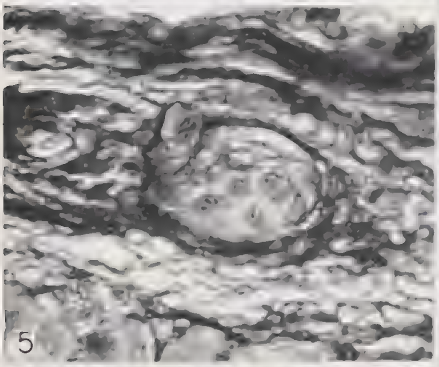
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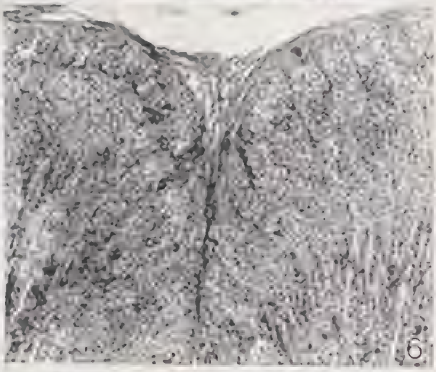
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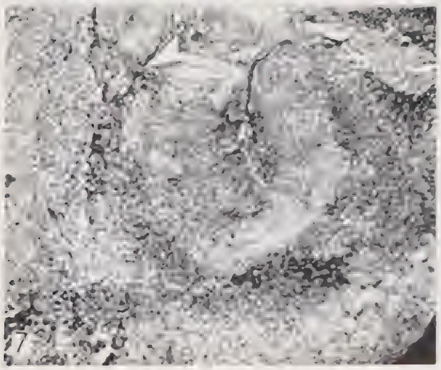
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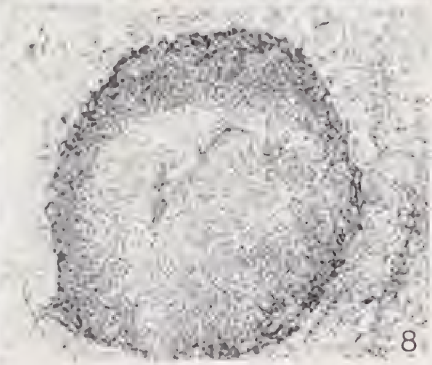
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## PLATE 2

### EXPLANATION OF FIGURES

9 Cell sequence in the adrenal gland of a frog subjected to dehydration. Note gradual transition from elongated to typical glandular cells. Paraffin section, Mallory's triple stain. × 500.

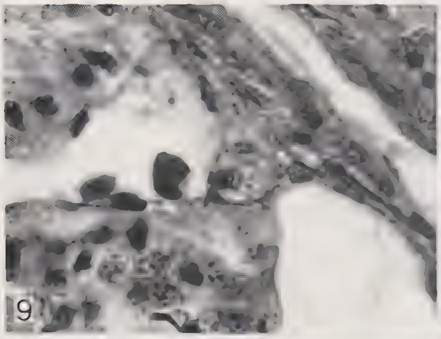
10 Accumulation of lipoid by differentiating capsule cells in a human adrenal gland. Gelatin-frozen section, Sudan IV-hematoxylin. × 1000.

11 Large outer fascicular cells of a cat. Note protein network and chondriosome 'knots.' In the living gland the spaces are filled with lipoids. Paraffin section, hematoxylin-eosin. × 2000.

12 Human fascicular cells with small fat droplets fairly uniformly distributed. Gelatin-frozen section, Sudan IV-hematoxylin. × 1000.

13 Transition between outer and inner fascicular cells. Paraffin section, Masson's trichrome. × 500.

14 Reticularis zone of the cat. Paraffin section, Masson's trichrome. × 500.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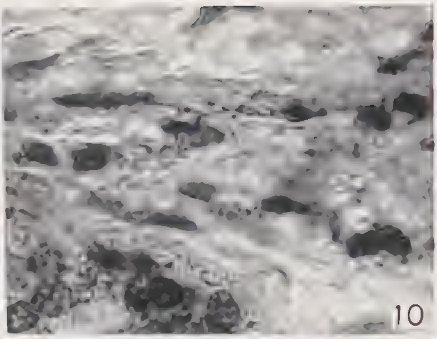
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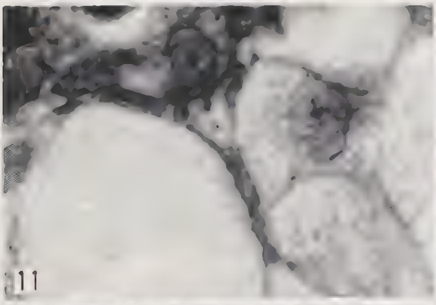
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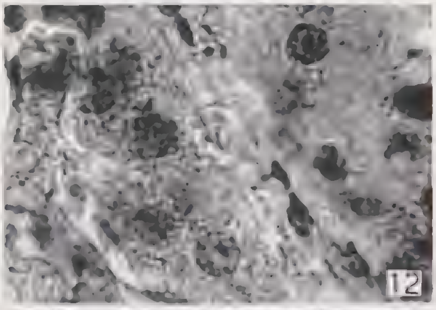
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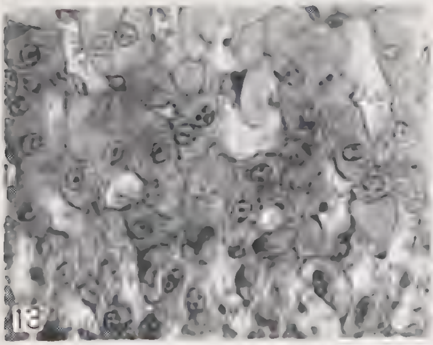
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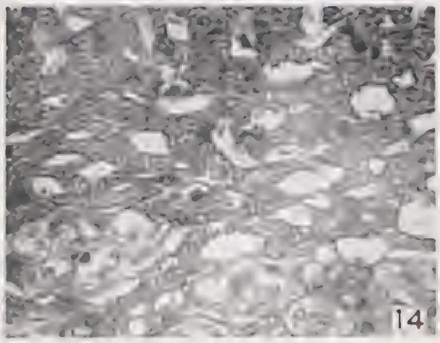
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# THE PHARMACOLOGY AND ANATOMY OF THE HYPOPHYSIS OF THE ARMADILLO[1,2]

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TWO PLATES (FOUR FIGURES)

## INTRODUCTION

This report contains an embryological, anatomical and pharmacological analysis of the hypophysis of the nine-banded armadillo (Dasypus novemcinctus) with particular reference to the site of elaboration of the neural lobe hormones.[2,3] This question has been the center of a lively controversy since Oliver and Schäfer (1895) first demonstrated pharmacological activity in extracts of the gland. As secretory granules were not found in the cells of the pars nervosa, investigators were long unwilling to ascribe a secretory role to the neuroglia-like cells, which are the only cells regularly found in significant numbers in this portion of the gland. The association between the lobes is frequently so intimate that many workers, especially in the clinical fields, believe that the oxytocic and pressor

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[1] Submitted in partial fulfillment of the requirements for the degree of doctor of philosophy at The University of Chicago. Acknowledgment is made of a grant from the Rockefeller Foundation which made this work possible.

[2] A preliminary report of this work was given at the April, 1938 meeting of the American Society for Pharmacology and Experimental Therapeutics.

[3] The following terminology will be used. The derivatives of the buccal ectoderm and of the neural ectoderm will be referred to as the pars buccalis and the pars nervosa respectively. The pars buccalis consists of the anterior lobe, pars tuberalis and pars intermedia (intermediate lobe). The pars nervosa consists of the neural lobe and the neural stalk. The term 'stalk' refers to that area between the neural lobe and the brain which was removed for pharmacological assay. This portion includes the neural stalk, the pars tuberalis and a variable amount of brain.

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hormones are formed, along with the melanophore-dispersing hormone, by the glandular cells of the pars intermedia or from their degradation products, and diffuse into the pars nervosa, appearing there in the form of colloid droplets, the so-called Herring bodies. This view is summarized by Rolleston ('36).

In the past few years, studies along widely divergent lines have indicated that the oxytocic, pressor and anti-diuretic hormones are formed by the pars nervosa. The tissue culture experiments of Geiling and Lewis ('35) showed that growths of the pars intermedia of the rat and mouse possess melanophore-dispersing, but no pressor activity. Cultures of neural lobe admixed with a small amount of pars intermedia had both pressor and melanophore-dispersing activity. Similarly, Anderson and Haymaker ('35) found melanophore-dispersing but no oxytocic or anti-diuretic activities in intermediate lobe growths.

Fisher, Ingram and Ranson ('38) in an extensive series of experiments produced diabetes insipidus in cats by means of lesions in the supraoptico-hypophyseal tracts and found that the hypophyses of these animals showed pronounced atrophic changes accompanied by a loss of oxytocic and pressor activity. The melanophore-dispersing activity was, however, unaffected and the intermediate and anterior lobes were unaltered histologically.

Gersh and Tarr ('35) using the Altmann-Gersh freezing-drying technique concluded that the Herring bodies are fixation artefacts arising from the evenly distributed albumin-like protein substance which infiltrates the pars nervosa. By comparison of the extractibility of this material and of the pressor hormone, they claimed the two are unrelated.

The most convincing proof of the role of the pars nervosa in the elaboration of the oxytocic, pressor and anti-diuretic hormones was advanced by Geiling and his associates (DeLawder, Tarr and Geiling, '34; Geiling, '35; Wislocki and Geiling, '36) through their investigations of the hypophysis of the whale and the chicken and more recently on the manatee

(Oldham, McCleery and Geiling, '38). They showed that although in these species an anatomically discrete pars intermedia is lacking and cells of the pars intermedia type are absent from the pars nervosa, pressor and oxytocic hormones are present in neural lobe extracts. The melanophore-dispersing hormone, on the other hand, is present only in anterior lobe extracts.[4] Since the anterior and neural lobes are clearly separated by connective tissue, passage of the hormones from one lobe to the other is improbable. Furthermore, the studies of Wislocki and King ('36) and Wislocki ('37 a, b) on the blood supply of the hypophysis show that the anterior and neural lobes have an independent blood supply and that venous drainage is such that interchange of secretions by this route is unlikely. The lymphatic drainage of the several parts of the hypophysis does not seem to have been worked out.

While the hypophyses of the whale and the chicken have furnished interesting results, they have certain disadvantages for experimental purposes. The small gland in the chicken is buried in a deep sella turcica and is difficult to approach. For unavoidable reasons, the whale pituitary could not be removed until 8 to 24 hours after the death of the animal, so that post-mortem changes were inevitable. Search was made, therefore, for another species in which an intermediate lobe was not present. A preliminary survey by M. R. Lewis of the Carnegie Institution of Embryology showed that the armadillo hypophysis answered this description, and with her acquiescence a detailed study of the pharmacology and anatomy of this gland was undertaken.[5]

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[4] Three or four active substances can be demonstrated in commercial extracts of the posterior pituitary, namely the pressor (anti-diuretic), oxytocic and melanophore-dispersing hormones. At present it is generally agreed that the pressor and anti-diuretic activities are mediated by the same hormone. Furthermore, it has been conclusively shown that the melanophore dispersing hormone arises in the pars buccalis. Accordingly in this paper, the term 'neural lobe hormones' refers to the pressor (anti-diuretic) and oxytocic hormones.

[5] During the course of preparation of this manuscript, there appeared a paper by Wislocki ('38) containing a description of the topography of the armadillo hypophysis. His findings accord well with the more detailed observations and experiments reported here.

The armadillo proved to be an ideal animal for this purpose. The animals are relatively inexpensive and can be obtained at short notice any time of the year; they can be kept in captivity for short lengths of time. The hypophysis is comparatively large and the lobes are easily approached and separated.

## ANATOMY OF THE ARMADILLO HYPOPHYSIS

### *Material and methods*

The armadillo is a member of the Edentata (Zenarthra), one of the lower orders of mammals. The animals used in this study (Dasypus novemcinctus) came from Texas. Glands were obtained from males and from pregnant and non-pregnant females as well as from embryos. While the age of the adult animals was not known, two of them were quite young, since the sphenoid bone was incompletely ossified. Embryos of the following crown-rump were obtained: 3.4 cm., 4.5 cm., 5 cm., 5.8 cm. and 7.7 cm. and a newly born animal of about 10 cm. Although the early stages are missing from this series, the material on hand permits an investigation of the fate of Rathke's pouch and the general development of the gland.

The armadillos were killed with ether, and the glands fixed immediately in formol-Zenker. A small amount of hypothalamus was left attached to the infundibular stalk in order to ensure complete removal of the pars tuberalis. In several cases, a portion of the underlying bone was removed together with the gland. After fixation, the material was embedded in nitrocellulose, serially sectioned at 8 μ and stained by the hematoxylin-eosin-azure II and the Mallory-azan methods. The glands were cut in either the sagittal, horizontal or frontal planes. Decalcification, when necessary, was done with 3% $HNO_3$ subsequent to embedding. Certain other histological methods were used. Bodian's ('36) colloidal silver method was used as a nerve fiber stain. This was not successful when tried on nitrocellulose-embedded material. Satisfactory results were obtained, however, when paraffin was used. Penfield's ('28) modification of the del Rio Hortega silver carbonate stain was used for impregnating the neuroglia cells of the pars nervosa. One gland was fixed in alcohol and stained with thionin blue for mast cells. Twenty-two glands were used for histological study and twenty-one for pharmacological assay.

## *Gross anatomical observations*

The armadillo hypophysis is a roughly oval structure lying in a shallow depression on the superior surface of the sphenoid bone. The long axis of the gland lies transversely to that of the body and measures approximately 0.9 cm. The anteroposterior diameter is approximately 0.6 cm. In most instances, the meninges over the neural lobe are dark because of the melanophores in them. In the fresh condition the somewhat triangular neural lobe can be distinguished by its very white appearance. It occupies an excavation in the posterior and dorsal surfaces of the gray-tan anterior lobe. The neural lobe is attached to the neural stalk which extends anteriorly and dorsally to join the hypothalamus. On either side of the junction of the infundibular stalk and the neural lobe, projections of anterior lobe tissue extend partially around each side of the pars nervosa and nearly meet in the midline of its dorsal surface. The weights of the two lobes of twenty-one armadillos are recorded in tables 1 and 2. The anterior lobe is roughly four times as heavy as the neural lobe.

TABLE 1

*Weight in milligrams of the separate portions of the hypophysis*

| | APRIL, 1936[1] | | | JANUARY, 1937[2] | | |
|---|---|---|---|---|---|---|
| | Ant. lobe | Post. lobe | Stalk | Ant. lobe | Post. lobe | Stalk |
| | 40.30 | 9.46 | 6.20 | 46.00 | 8.25 | ... |
| | 44.90 | 9.00 | 1.90 | 22.50 | 7.00 | ... |
| | 36.40 | 6.64 | 3.51 | 38.00 | 8.00 | 2.85 |
| | 36.50 | 6.94 | 4.10 | 29.00 | 8.30 | 4.11 |
| | 34.50 | 8.70 | 5.30 | 24.00 | 8.90 | 0.90 |
| Total | 192.60 | 40.74 | 21.01 | 159.50 | 40.45 | 7.86 |
| Average | 38.52 | 8.15 | 4.20 | 31.90 | 8.09 | 2.62 |

[1] Sex not recorded.

[2] Male animals.

The gland is embedded in a fairly thick dural sheath. An extension of this dense connective tissue completely separates the two lobes. An obvious subdural space is not apparent. The connections between this connective tissue and the lobes are fairly loose and the neural lobe can be lifted away from the anterior lobe by gross dissection. This is facilitated by the use of a low power dissecting microscope. Neither a pars tuberalis nor a pars intermedia is visible on gross inspection.

TABLE 2

*Weight in milligrams of the separate portions of the hypophysis*

| | WEIGHT OF SEPARATED PORTIONS IN MILLIGRAMS | | | BODY WEIGHT IN KILOGRAMS |
|---|---|---|---|---|
| | Ant. lobe | Post. lobe | Stalk | |
| November, 1937 [1] | 43.40 | 6.41 | 3.76 | 4.45 |
| November, 1937 [1] | 29.40 | 8.55 | 0.68 | 3.37 |
| November, 1937 [1] | 16.80 | 4.52 | ... | 1.72 |
| November, 1937 [1] | 34.60 | 10.10 | 4.78 | 4.00 |
| November, 1937 [1] | 24.40 | 8.72 | 4.46 | 4.20 |
| November, 1937 [1] | 31.40 | 10.10 | 3.50 | 3.90 |
| November, 1937 [1] | 34.40 | 8.20 | 4.65 | 2.62 |
| November, 1937 [1] | 43.80 | 11.60 | ... | 3.90 |
| Total | 258.20 | 68.20 | 21.83 | 28.16 |
| Average | 32.27 | 8.52 | 3.64 | 3.52 |
| February, 1938 [2] | 25.50 | 7.96 | 10.90 | 2.97 |
| February, 1938 [2] | 37.90 | 7.51 | ... | 3.90 |
| February, 1938 [2] | 43.70 | 8.16 | 7.51 | 3.45 |
| Total | 107.10 | 23.63 | 18.41 | 10.32 |
| Average | 35.70 | 7.88 | 9.21 | 3.44 |

[1] Female animals killed by electrocution.

[2] Female animals.

## *Histological findings*

*General observations.* The distinctive microscopic feature of the armadillo hypophysis is the absence of a discrete pars intermedia. The anterior and neural lobes are completely separated by connective tissue and no cells of the type found in the pars intermedia of most animals can be seen in the neural lobe. The clear-cut separation of the two lobes is particularly well seen in sections stained with the Mallory-azan method. In them, a layer of dense collagenous connective tissue of variable thickness is apparent as a deeply blue staining septum.

*Anterior lobe.* The anterior lobe of the armadillo hypophysis consists of nests of closely packed chromophobe and chromophil cells supported by a network of reticular connective tissue. Posteriorly the meshes of the connective tissue are finer and the cells are more closely packed. Numerous irregular blood sinuses are present in this connective tissue. A drop of colloid may be seen frequently in the center of a nest of cells. The relative number and the distribution of the various types of anterior lobe cells varies widely in the different glands and in different regions of the same gland.

The predominant cell in the anterior lobe of the armadillo hypophysis is the alpha cell. With the hematoxylin-eosin-azure II stain, the cytoplasm of these cells contains a varying number of small eosinophilic granules with frequently a lighter staining area to one side of the nucleus. In the Mallory-azan preparations the granules of some of the cells stain with azo-carmine while in others they stain with orange-G. The spherical nucleus contains a central nucleolus and a varying number of large chromatin particles at the pheriphery and finer ones scattered through the nucleus. In some of these cells, the nucleus stains darkly and is less regular in outline.

The beta cells are scarce in the armadillo hypophysis. With hematoxylin-eosin-azure II, the cytoplasm contains pale blue granules. Since the granules are very fine, the beta cells are hard to distinguish from the chromophobes with this stain. With the Mallory-azan stain, the granules are a more intense blue. The cytoplasm is frequently vacuolated and the irregularly-shaped nuclei stain darkly and appear pycnotic. The negative image of the Golgi apparatus is usually clearly seen in the Mallory-azan preparations.

Two main types of chromophobic cells can be distinguished. One type is found scattered among the chromophilic cells. The non-granular cytoplasm is usually small in amount and stains a faint blue with hematoxylin-eosin-azure II. The nuclei are similar to those of the alpha cells. The second type of chromophobe is usually found in nests. The nucleus appears shrivelled and stains intensely. Faint basophilic strands divide the cytoplasm into compartments which have the appearance of large vacuoles. A number of forms intermediate between chromophils and chromophobes may be seen.

An unusual type of cell was seen in the anterior lobe of all the armadillo hypophyses examined. These cells are easily distinguished by their size and staining properties from the chromophobe and chromophil glandular cells and from the connective tissue elements. Their diameter is from one and a half to three times that of the acidophils. At first glance they are somewhat suggestive of sympathetic ganglion cells. Neurofibrils have not been demonstrated in them; occasionally the cytoplasm contains large basophilic particles which stain intensely with the hematoxylin-eosin-azure II and are somewhat suggestive of irregular Nissl bodies. The peripheral cytoplasm is usually clear and the cell limits hard to define. A layer of cytoplasm staining a faint blue with hematoxylin-eosin-azure II surrounds the nucleus and extends irregularly into the cytoplasm. The diameter of the large nucleus is about twice that of the average glandular cell. It is usually situated at one side of the cell and may be round or oval in outline and is occasionally slightly indented. There is a single prominent nucleolus (or rarely two or more), irregular in outline and usually eccentric in position. In many of the cells, small particles of chromatin are scattered throughout the nucleus. In others, there are finely granular linin strands with an occasional chromatin particle. These cells are found mainly on each side of the mid-line in the anterior two-thirds of the anterior lobe and usually in its dorsal half. They are most numerous in the region where the anterior lobe and the pars tuberalis are in contact. They are found there in small groups and the individual cells are often partially surrounded by connective tissue. More anteriorly in the anterior lobe the cells are in general smaller and less conspicuous.

Colloid-filled clefts are seen in the posterior portion of the anterior lobe. These are seen best in frontal and horizontal sections and may be quite extensive. They are usually lined by a flattened epithelium, although occasionally by a ciliated, cuboidal epithelium. The cells between these clefts and the juxta-neural border of the anterior lobe are similar to those found elsewhere in the lobe.

*Pars tuberalis.* The extensive pars tuberalis consists of a sheet of cells several layers in thickness surrounding the stalk and extending under the hypothalamus both anteriorly and posteriorly, the anterior extension being the more prominent. Most of the cells are chromophobes, although occasionally granular acidophils are present. The cells are arranged characteristically in small follicles in the center of which are colloid droplets. There is an extensive and well-vascularized connective tissue framework which permeates the lobe and extends as a continuous layer separating the pars tuberalis from the neural lobe. Ventrally, the transition between the anterior lobe and the pars tuberalis is a gradual one and the two cannot be separated.

*Neural lobe.* The neural lobe of the armadillo hypophysis contains more cells and more Herring bodies than are seen in most other species. The blood supply is rich and there is much perivascular connective tissue which in sections is made prominent by the Mallory-azan stain. The infundibular recess extends down the stalk; isolated portions in the form of ependymal lined spaces are often visible in the neural lobe. These isolated portions, when traced through serial sections, are found to be independent of the main cavity. Preparations stained by Bodian's method for nerve fibers show numerous fibers streaming down from the hypothalamus and branching extensively in the neural lobe. The exact nature of these fibers and of their terminations in the armadillo remains to be determined.

The most common cell type in the neural lobe has a small irregularly-shaped and deeply stained nucleus containing several acidophil nucleoli. With the ordinary techniques, the long cytoplasmic processes of these cells cannot always be distinguished from the somewhat fibrillar background of the lobe. With Penfield's silver carbonate stain, these processes are made more prominent. Several processes, often with numerous branches, may arise from a single cell; in many cases they are in close contact and often seem to be continuous. They frequently end on blood vessels or on the connective tissue capsule of the lobe.

Another type of cell is present in considerable numbers and is conspicuous because of its large size. It is most frequently seen in the anterior and central portion of the lobe. These cells appear usually in groups and, occasionally, cytoplasmic membranes cannot be made out between the individual cells so that they appear multi-nucleated. The diameter of the nuclei of these cells is two to three times that of the length of the nuclei of the neuroglia-like cells. The nuclei of the large cells contain one or more prominent, relatively large nucleoli and a scattering of fine chromatin particles. With the hematoxylin-eosin-azure II stain, a fairly extensive, faintly or deeply basophilic and finely granular cytoplasm can be seen. There are numerous intermediate cells between the large and the neuroglia-like cells. In the connective tissue of the neural lobe, fibroblasts are common and are not readily distinguished from many of the neuroglia-like cells. Mast cells and plasma cells are present in small numbers.

Both melanoblasts and melanophores are seen in the majority of the glands. These cells appear to be confined to the meninges and enter the neural lobe only in association with blood vessels. They are seen in greatest numbers in the leptomeninges of the ventral surface of the hypothalamus and in the connective tissue of the pars tuberalis. Another common site is over the dorsal surface of the neural lobe and to a lesser extent of the anterior lobe. They are also present in the connective tissue separating the two lobes and occasionally in the connective tissue network of the periphery of the anterior lobe.

*Neural stalk.* The neural stalk is very vascular. The ependymal-lined infundibular recess occupies the central portion. Neuroglia-like cells and cells with large nuclei similar to those in the neural lobe are found to the side of the recess. Bodian's stain for nerve fibers shows fibers streaming down on either side of the recess.

## *Embryological findings*

The anterior lobe of the 3.4 armadillo embryo consists of loosely packed cells, separated by strands of connective tissue and large vascular sinuses. The majority of the cells are chromophobes, others have a faintly-staining non-granular cytoplasm. Projections of anterior lobe tissue extend posteriorly and partially surround the neural stalk. A large cleft occupies the posterior portion of the lobe, and also extends into the posteromedial projections of the lobe. The posterior border of this cleft is composed of several irregularly arranged layers of closely packed cells. The anterior border of the cleft is formed mostly by cell nests.

The neural lobe lies posterior to the anterior and is separated from the latter by a layer of collagenous connective tissue. The cells are seen in groups in the central portion of the lobe and are of two types. In one the nucleus is relatively small, roughly oval and stains fairly deeply. The nucleus of the other type is larger, vesicular and usually spherical. The neural lobe is connected with the brain by the neural stalk, which contains the ependymal-lined infundibular recess.

The hypophyses of the 4.5- and the 4.8-cm. embryos are similar. The cell nests in the anterior lobe are more closely packed than in the 3.4-cm. embryo. The more deeply staining cells now appear to be the predominant type, although chromophobes are also abundant. The cleft is still conspicuous. The cells composing its posterior border seem to be less closely packed, and the chromophobic cytoplasm can be seen in many of the cells.

In the neural lobe many of the cells with the small nuclei appear to be migrating toward the periphery of the lobe.

Long cytoplasmic processes extend from these cells. The cells with the larger, more vesicular nuclei tend to remain in the center of the lobe.

The 5-cm. and the 5.8-cm. embryos are essentially the same. There is a considerable increase in the size of the anterior lobe both laterally and anteriorly. Occasional faintly granular alpha cells are seen for the first time in the anterior lobe. There is a great increase in the number of cells in the neural lobe, specially those with the small nuclei. Mitoses are frequent. Melanophores are seen in the connective tissue surrounding the hypophysis of the 5-cm. embryo.

The hypophysis of the 7.7-cm. embryo approaches closely the form of the adult gland. The anterior lobe is flattened dorsoventrally and the neural lobe lies on its dorsal and posterior surfaces. In mid-sagittal sections, only a small narrow cleft can be seen. More laterally, however, it is still fairly extensive.

In the newborn animal (approximately 10 cm.) the cleft is present as a narrow slit in the posterior and ventral portion of the gland. It is absent from the posterior extensions of the anterior lobe. Alpha cells are now numerous and deeply staining.

In all the embryos studied, the anterior and neural lobes are clearly separated by connective tissue and anterior lobe cells are never seen in the pars nervosa. Colloid droplets are not seen in the anterior lobe or Herring bodies in the neural lobe.

## PHARMACOLOGY OF THE ARMADILLO HYPOPHYSIS

### *Material and methods*

The glands were separated into three parts: anterior lobe, neural lobe and 'stalk.' The animals were anesthetized deeply with ether or stunned by electrocution. The skull was opened and the brain removed except for a small portion which was left attached to the neural stalk. The meningeal covering of the shallow sella was cut away, exposing the dorsal surface of the gland. The neural lobe was then lifted up and separated from the stalk. Care was taken to make this separation sufficiently posterior to avoid inclusion of a portion of the pars tuberalis in the neural lobe. In spite of this precaution, serial sections of the separated portions of one gland show a few nests of pars tuberalis cells attached to the neural lobe. The neural stalk and the pars tuberalis which surround it, were removed along with a small portion of the brain attached to the stalk. Occasionally the connection between the neural stalk and the brain was accidentally severed and in these cases no brain tissue was removed. The connective tissue which separated the neural and anterior lobes was stripped aside and the anterior lobe was lifted away from the underlying connective tissue.

As the three portions were removed, they were weighed on a torsion balance and placed immediately into acetone. The weights of the individual portions are incorporated in tables 1 and 2. The variations in the weights of the 'stalk' can be readily explained by the impossibility of distinguishing this portion from the neural lobe on one hand, and the brain on the other, and because excess of brain tissue was usually included with the stalk in order to ensure complete removal of the pars tuberalis.

The separated and acetone-desiccated portions were then extracted with 0.25% acetic acid according to the specifications set forth in the United States Pharmacopoeia XI for the preparation of standard pituitary. Since the individual lobes are so small, it was found advisable to combine those of a small group of animals rather than to attempt to assay each gland separately. The extracts were assayed for pressor, anti-diuretic, oxytocic and melanophore-dispersing activities. All assays are expressed in terms of the international standard.

## *Pharmacological findings*

*Pressor hormone.* The pressor activity was assayed by the blood-pressor raising effect on the phenobarbital-anesthetized cat, according to the recognized technique. The pressor activity of the armadillo neural lobe extracts was found to be equivalent to that of the standard powder (fig. 3). The anterior lobe extracts have little or no pressor effect. Extracts of the 'stalk' contain considerably less activity than those from the neural lobe. However, they show a definite pressor effect, whereas the rise of blood pressure with the anterior lobe extracts is so slow and prolonged that it is apparently due to impurities or possibly to an accidental admixture with the active neural lobe.

*Anti-diuretic hormone.* The anti-diuretic activity was measured by a modification of Burn's ('31) technique, which in turn is an adaptation of Gibb's ('30) method. The results summarized in table 3 show that the anti-diuretic substance in the armadillo hypophysis is confined to the neural lobe and

TABLE 3

*Comparison of anti-diuretic activity of the anterior and posterior lobes of the armadillo hypophysis*

| | CONTROL | STANDARD | POST. ARM. | ANT. ARM. |
|---|---|---|---|---|
| Group I | 120.0 | 186.0 | 198.0 | 118.0 |
| Group II | 112.0 | 194.0 | 199.0 | 111.6 |
| Group III | 108.0 | 191.0 | 214.0 | 106.0 |
| Group IV | 97.5 | 202.5 | 213.0 | 124.0 |
| Average | 109.4 | 193.4 | 206.0 | 114.9 |

Each animal received 10 cc. of tap water at 37°C. intraperitoneally; and subcutaneously, 0.5 cc. of dilute pituitary solution (0.25 cc. of extract, 1 mg. per cubic centimeter, diluted with saline to 5 cc.).

that its activity in this species is approximately that of the standard power.

*Oxytocic hormone.* Assays by the standard technique employing the virgin guinea pig uterus show consistently that the oxytocic content of the neural lobe of the armadillo hypophysis is only about 10% that of the standard (fig. 4). Assays by the chicken blood pressure method (Coon, '38), however, show this activity to be about 30% that of the standard. Results by the two methods of assay regularly show such a discrepancy when there is a great preponderance of the pressor hormone (Coon, personal communication). Extracts of the anterior lobe and of the 'stalk' showed by both methods of assay, only traces of oxytocic activity.

*Inactivation of the pressor and oxytocic hormones by alkali.* In order to see whether any of the observed pressor or oxytocic effects of the armadillo hypophysis were due to impurities, the hormones were destroyed by alkali. To 0.5 cc. of neural extract was added 0.25 cc. 2 N NaOH and the mixture left overnight. It was then neutralized with 1 N HCl and the volume made up to 2 cc. with 0.25% acetic acid. Following this treatment, no pressor or oxytocic response could be elicited showing that the previous effects with unalkalinized preparations were due to the hormones and not to impurities.

TABLE 4

*Melanophore activity of the anterior and neural lobes of the armadillo compared with the standard powder*

| TIME AFTER INJECTION (IN MINUTES) | ARM. POST. LOBE 0.3 MG. | ARM. POST. LOBE 0.06 MG. | ARM. ANT. LOBE 0.3 MG. | ARM. ANT. LOBE 0.03 MG. | ARM. ANT. LOBE 0.003 MG. | STANDARD POWDER 0.03 MG. | STANDARD POWDER 0.003 MG. |
|---|---|---|---|---|---|---|---|
| 30 | ? | 0 | ++ | ++ | 0 | ++ | — |
| 60 | + | 0 | +++ | ++ | ? | ++ | ++ |
| 120 | ? | 0 | +++ | +++ | 0 | +++ | ++ |
| 150 | 0 | 0 | ++ | — | 0 | — | + |
| 180 | 0 | 0 | ++ | — | 0 | — | — |
| 210 | 0 | 0 | ++ | + | 0 | + | 0 |
| 240 | 0 | 0 | ++ | + | 0 | + | 0 |

The extracts were diluted with 0.25% acetic acid so that 0.25 cc. contained the desired amount of material. Injection was made into the ventral lymph sac. Hypophysectomized frogs were used throughout. The degree of darkening of the frog is expressed as + (slight), ++ (moderate) and +++ (maximal).

*Melanophore-dispersing hormone.* Hypophysectomized frogs (Rana pipiens) were used for the assay of the melanophore-dispersing hormone, since it has been shown that in such animals the action of this hormone is apparently a specific one (Teague and Noojin, '38). These assays showed regularly the presence of the melanophore-dispersing hormone in the anterior lobe extracts with only traces in the neural lobe and 'stalk.' While this method of assay is not a very satisfactory one for quantitative results, nevertheless comparison of the activity of the anterior lobe preparations with that of the standard powder showed the latter to be about ten times stronger (table 4).

## DISCUSSION

The distribution of the pressor, anti-diuretic, oxytocic and melanophore-dispersing hormones in the armadillo hypophysis indicates that the first three of these principles are formed in the pars neuralis and the last in the anterior lobe.

Various investigators (see summary by Atwell and Holley, '36) have shown that in many species, the intermediate lobe of the pars buccalis is responsible for the elaboration of the melanophore-dispersing substance. In most animals, the intermediate and neural lobes are in such intimate contact that complete separation of the two is impossible, so that the melanophore-dispersing hormone is found in posterior lobe extracts along with the pressor, anti-diuretic and oxytocic hormones. This led to the belief that these latter hormones might also arise in the intermediate lobe. In the armadillo hypophysis, however, there is no discrete intermediate lobe and cells of the type found in the intermediate lobe of other species are not present in the pars nervosa. Furthermore, the pars buccalis and the pars nervosa are clearly separated by connective tissue. In addition, assays of the separated lobes show clearly that the oxytocic, pressor and anti-diuretic hormones are present in the neural lobe extracts, while only traces of the melanophore-dispersing hormones are present in such extracts. On the other hand, the anterior lobe extracts are rich in melanophore-dispersing hormone but contain only traces of the oxytocic and pressor hormones, which are in all probability contaminants. Hence it appears that the neural lobe hormones are not elaborated by the cells that give rise to the melanophore-dispersing hormone, but are formed in the pars nervosa.

Absence of a pars intermedia has been previously reported in birds (de Beer, '26), the porpoise (Wislocki, '29), whale (Valso, '34; Geiling, '35; Wislocki and Geiling, '36) and manatee (Oldham, McCleery and Geiling, '38), but a satisfactory explanation for its absence in these species has not been advanced. Except in the birds, the embryonic development of the hypophysis has not been studied in any of these species. Bruni ('14) and de Beer ('26) draw attention to the early closure of Rathke's pouch in the bird. However, in all the armadillo embryos examined, the pouch extends along the juxtaneural region of the anterior lobe and, in all cases, the anterior and neural lobes are closely associated although completely separated from one another by a layer of collagenous connective tissue. In this species, therefore, the absence of a discrete pars intermedia cannot be attributed to an early closure of Rathke's pouch.

It has been suggested that the formation of the intermediate lobe depends upon contact of the pars buccalis with the brain. Atwell and Holley ('36) extirpated the caudal region of the hypophyseal anlage in tadpoles and obtained silvery animals that underwent complete metamorphosis. Histological examination of the gland showed that the intermediate lobe was absent and that the anterior lobe was at some distance rostral to the neural lobe. These findings, supplemented by transplantation experiments (Atwell, '35), led to the suggestion that contact of the epithelial anlage with the brain floor may be necessary for the early development of the intermediate lobe. Gaillard ('37) grew explants of the anterior and posterior lobes in tissue culture and showed that only when the two lobes were in contact did an intermediate lobe-like structure appear. He suggested that the structure of the intermediate lobe and of the pars tuberalis may be due to a direct influence of the pars nervosa and infundibular body upon the pars oralis lying in contact with them. However, since in the armadillo, chicken (DeLawder, Tarr and Geiling, '34), whale and manatee, the intermediate lobe substance can be demonstrated in anterior lobe extracts, and since a discrete intermediate lobe does not exist in these animals, cells having a secretory function similar to that of the intermediate lobe cells of other species are evidently present in the anterior lobe of these animals. These cells are probably scattered among the anterior lobe cells and are indistinguishable from them by the staining methods employed.

There is no obvious phylogenetic significance to the absence of a pars intermedia. While this lobe is apparently lacking in all birds, it is well developed in the reptiles (de Beer, '26). Although all Cetacea examined have no intermediate lobe, it is present in two other members of the Edentata, namely the sloth and the anteater (Wislocki, '38). It is also present in the Marsupialia (Parker, '17). Aquatic mammals of higher orders such as the seal (Wislocki and Geiling, '36) and the walrus (Oldham, unpublished observations[6]) have a well-developed intermediate lobe.

The low oxytocic content of the armadillo hypophysis is of considerable interest. A similar discrepancy between oxytocic and pressor content has been found in the glands of the finback, blue, humpback and sperm whales (Geiling, '35; McClosky, Miller and LeMessurier, '36). In the case of the whale, however, the glands could not be removed until 8 to 24 hours after the death of the animals, so that a postmortem loss of activity is possible. On the other hand, the armadillo glands were fixed immediately after the death of the animal. The difference in hormone content indicates that in this species, the oxytocic and pressor principles are separate entities. This is in contrast with the reports of Simon ('34) and Simon and Kardos ('34) in the hypophyses of the rat, cat, guinea pig and rabbit. In these animals, the oxytocic and pressor contents were found to run parallel. Furthermore, if the animals were kept on a water-free diet, there was a decrease in both principles.

The absence of appreciable amounts of the melanophore-dispersing hormone in 'stalk' extracts shows that the pars tuberalis, which is extensively developed in the armadillo, has not assumed the function of the intermediate lobe in this animal. The presence of both oxytocic and pressor activity is to be expected in view of the similar histological structure of the neural stalk and neural lobe.

The large colloid-filled clefts seen in the posterior portion of the anterior lobe may possibly be remnants of Rathke's pouch. Similar clefts have been reported in various other animals and are considered by some as being pinched off diverticula of the pouch. Kirkman ('38), in a study of the guinea pig hypophysis, considers these have a physiological rather than an embryological significance. It is felt that in the armadillo their location and the constancy of their appearance indicate that they are remnants of the pouch.

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[6] This gland was kindly supplied by Dr. George Crile of the Cleveland Clinics.

The alpha and chromophobe cells of the anterior lobe of the armadillo hypophysis correspond closely to similar cell types in other species. The beta cells, however, appear in limited numbers and because of their deeply-staining, irregularly-shaped nuclei and frequently vacuolated cytoplasm, they are suggestive of degenerating cells. With the techniques employed, neither type of granular cell stains very satisfactorily and it is possible that cells considered here as chromophobes are actually finely granular beta cells. Bryant ('30) reported a similar difficulty in distinguishing between these two types in the rabbit hypophysis, and Smith and McDowell ('30) were unable to demonstrate basophils satisfactorily in the hypophysis of the mouse.

The large cells seen in the anterior lobe of the armadillo hypophysis have apparently not been described in other species, although a somewhat similar cell was seen in the anterior lobe of the manatee hypophysis. However, in the case of the manatee, only one gland was available for study and this was not fixed until some 5 hours after the death of the animal. Wolfe, Bryan and Wright ('38) mention that large cells appear frequently in old rats. The large cells in the armadillo hypophysis were seen in the day-old specimen and in two animals in which the sphenoid bone was not completely calcified. The constancy of their distribution and the absence of intermediate forms speaks against them being merely swollen granular cells. They may represent an additional type of epithelial cell whose functional significance is as yet unknown. Although their large size and large vesicular nuclei are suggestive of sympathetic ganglion cells, neurofibrils have not been demonstrated. In some of the hematoxylin-eosin-azure II preparations, large azure-staining masses can be seen which are somewhat suggestive of Nissl substance. Elucidation of the nature of these cells requires a more detailed study of their cytology and embryogenesis.

Two types of cells are seen in significant number in the neural lobe of the embryo and adult armadillo. The cells with a small nucleus and long cytoplasmic processes are similar to those described by Bucy ('30) in the ox and called by him 'pituicytes.' Similar cells have been shown to exist in the neural lobe of many other species. They are considered by Bucy to be a special type of glial cell. The second type of cell may possibly be merely 'pituicytes' in a different stage of activity from those with the smaller nuclei. An alternative suggestion is that they are neuroblastic derivatives of the ependyma. The large vesicular nuclei and the cytoplasm staining deeply in some of the cells suggest somewhat the appearance of a nerve cell.

Gersh has recently described 'glandular' cells in the posterior lobe of many species. These cells have long cytoplasmic processes and contain granules which may be seen in the fresh tissue or in material fixed in Maximow's fluid and post-osmicated. He described changes in these cells during certain physiological conditions. While his methods have not been applied to the armadillo, the neuroglia-like cells correspond closely to Gersh's glandular cells and may be identical with them.

This work was carried out under the direction of Dr. E. M. K. Geiling of the department of pharmacology and Dr. William Bloom of the department of anatomy, whose helpful suggestions and criticism have been greatly appreciated.

## CONCLUSIONS

The distinctive microscopic feature of the armadillo hypophysis is the absence of a discrete intermediate lobe. The pars buccalis is completely separated from the pars nervosa by connective tissue and no pars buccalis cells can be seen in the pars nervosa.

Chromophobe and chromophil cells similar to those of other species are found in the anterior lobe of the armadillo. In addition, a large type of cell is present. Two main types of cells are present in the neural lobe. One is a neuroglia-like cell similar to those described in the neural lobe of other species. The nature of the second type of cell is not known.

The embryological findings indicate that in the armadillo the absence of a discrete intermediate lobe is not due to early closure of Rathke's pouch.

The melanophore-dispersing hormone is present in the anterior lobe extracts. Pressor, anti-diuretic and oxytocic hormones are present in neural lobe extracts. Traces of all four hormones are present in the 'stalk.'

The pressor and anti-diuretic activities of the neural lobe of the armadillo hypophysis are present in the same concentration as in the standard powder. The oxytocic content of the armadillo hypophysis is, however, only about 10% that of the standard powder.

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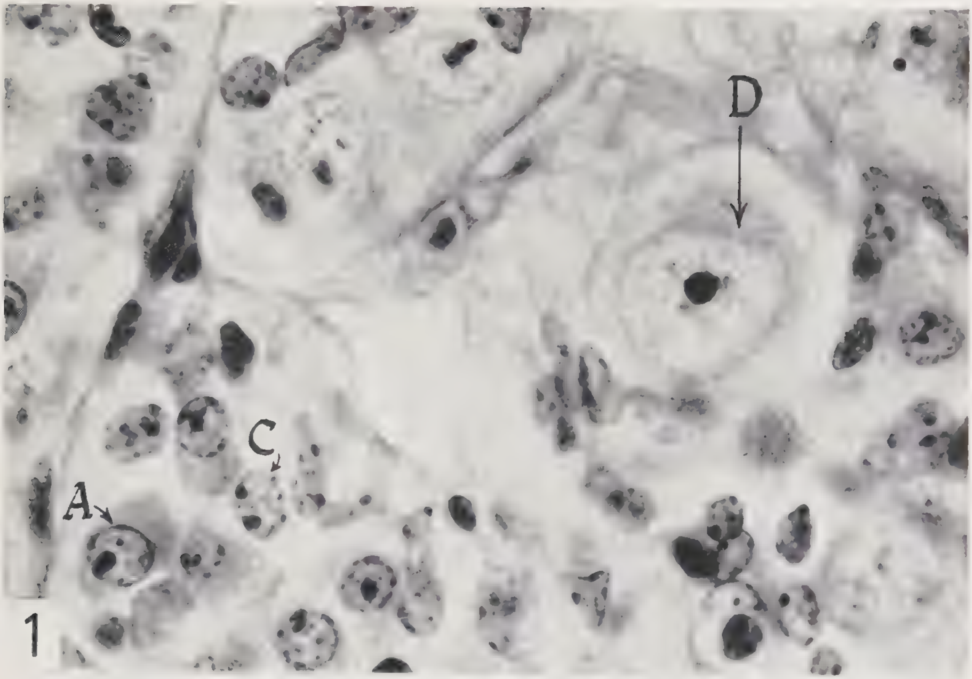
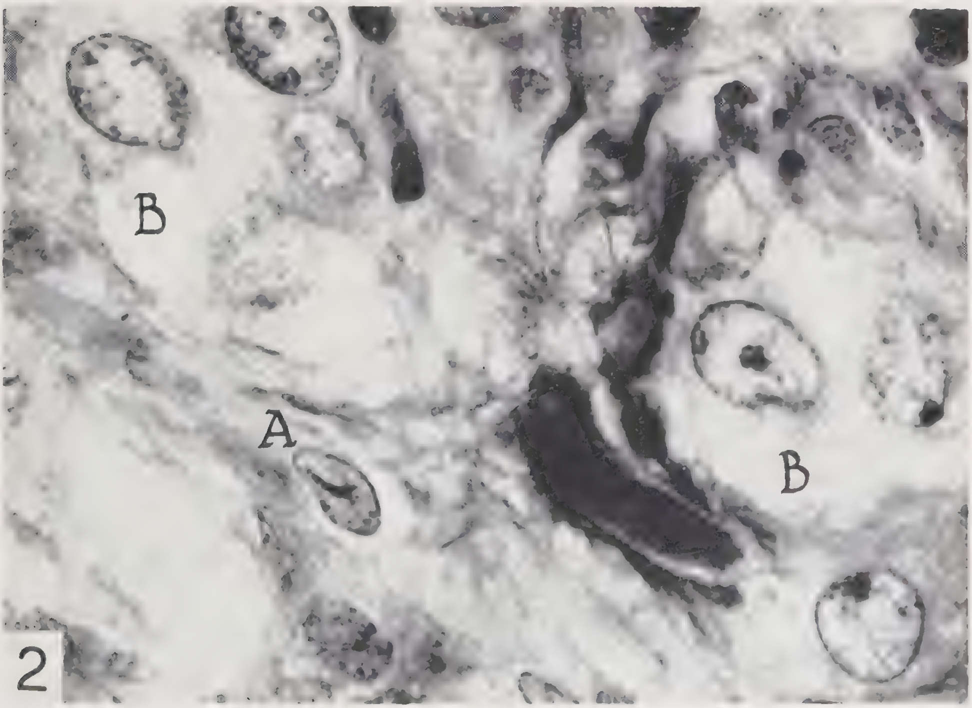
## PLATE 1

### EXPLANATION OF FIGURES

1 Armadillo hypophysis: anterior lobe, Zenker-formol, Mallory-azan. × 1225. D, large cell; A, acidophil cell; C, chromophobe cell.

2 Armadillo hypophysis: neural lobe, Zenker-formol, Mallory-azan. × 1225. A, neuroglia-like cell; B, group of cells with large vesicular nuclei.


D
C
A
1


B
A
B
2

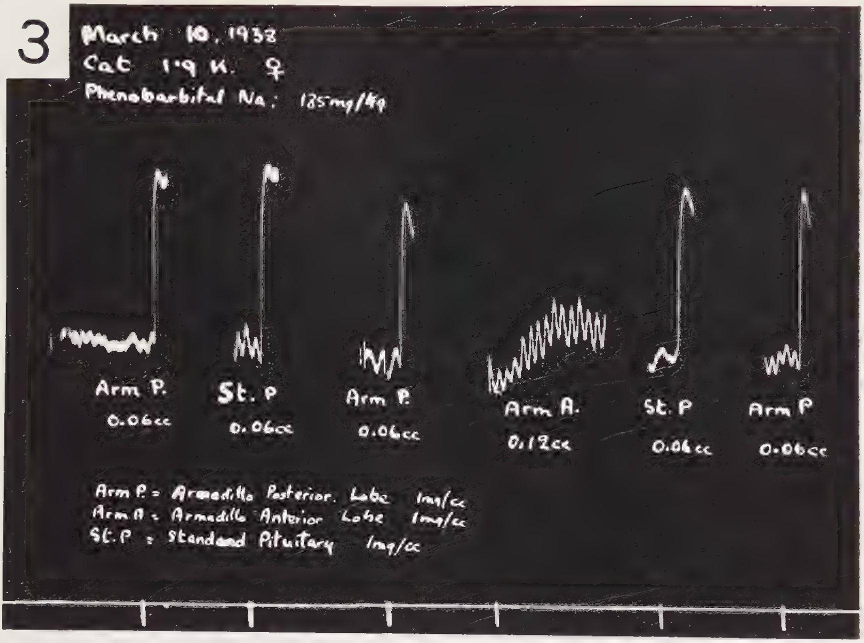
# PLATE 2

## EXPLANATION OF FIGURES

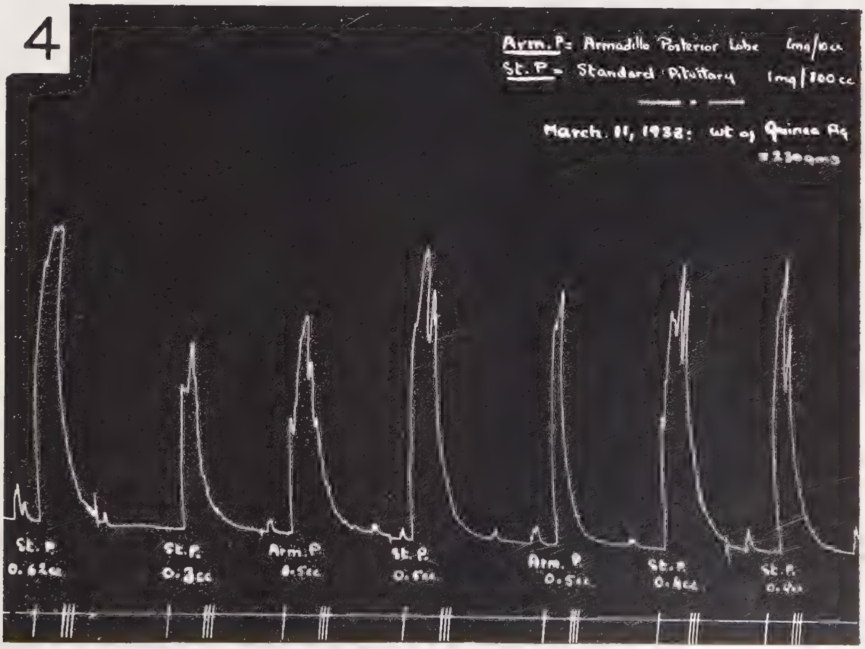
3 Blood pressure tracing; female cat 1.9 kg., sodium phenobarbital anesthesia, injections at approximately 30-minute intervals.

4 Contractions of uterus of virgin guinea pig, 230 gm. Arm. P, armadillo neural lobe, 1 mg. in 10 cc. distilled water; St.P., standard pituitary, 1 mg. in 100 cc. distilled water.

3


March 10, 1938
Cat 1·9 K. ♀
Phenobarbital Na: 185 mg/kg
Arm P.
0.06cc
St. P
0.06cc
Arm P.
0.06cc
Arm A.
0.12cc
St. P
0.06cc
Arm P
0.06cc
Arm P. = Armadillo Posterior Lobe 1mg/cc
Arm A = Armadillo Anterior Lobe 1mg/cc
St. P = Standard Pituitary 1mg/cc

4


Arm. P = Armadillo Posterior Lobe 1mg/10cc
St. P = Standard Pituitary 1mg/100cc
March 11, 1938: wt of Guinea Pig
= 230 gms
St. P.
0.6cc
St. P.
0.3cc
Arm. P.
0.5cc
St. P.
0.5cc
Arm. P.
0.5cc
St. P.
0.4cc
St. P.
0.4cc

# ACTIVE TESTICULAR EPITHELIUM IN THE CONNECTIVE TISSUE SURROUNDING A HUMAN SUPRANRENAL GLAND

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*New York Medical College and Flower Hospital, New York City*

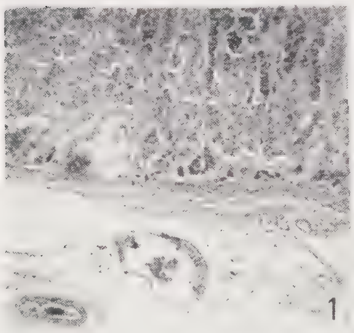
FOUR FIGURES

While studying a slide of the human suprarenal gland in the class in histology, the junior author found a tubule of testis in the connective tissue about the capsule. Other slides made from the same block of tissue revealed seventeen sections that contained evidences of the tubule. Some sections had evidently been lost, as a consecutive series of the tubules was not available. Additional tissue was cut from the gland on each side of the region occupied by the original block. Upon sectioning, no testicular tissue was found.

In the sections which contained the tubule, only a little connective tissue was present, and this had been considerably torn when the gland was removed at autopsy. Consequently it was suspected that the tubule might have been foreign to the suprarenal, and added to the block of tissue at some point in its preparation for histological study. However, upon careful examination of each section, complete continuity was demonstrable between tubule and suprarenal capsule, as shown in figure 1.

The testicular epithelium, though somewhat ragged, shows all stages of spermatogenesis except second spermatocytes. Spermatogonia, Sertoli cells, and prophases of first spermatocytes appear normal. A Sertoli nucleus is shown at a in figure 4. The two sperm heads there shown are associated with another Sertoli nucleus out of focus. This appears as a dark area above a. The tails of the sperm cells are not discernible. No cells in metaphase or anaphase have been found in the few sections available.

The tissue came from the pathology laboratory of the New York Medical College, and had been prepared for routine histological examination. The gland was removed at autopsy from a middle-aged male. Cause of death was reported as carcinoma of the lung with acute medullary choke. Histologically the suprarenal itself presents only slight abnormalities, these consisting chiefly of areas of focal hyperplasia in the zona reticularis. The zona glomerulosa is very thin in some places and in other parts extends deeply into the zona fasciculata.



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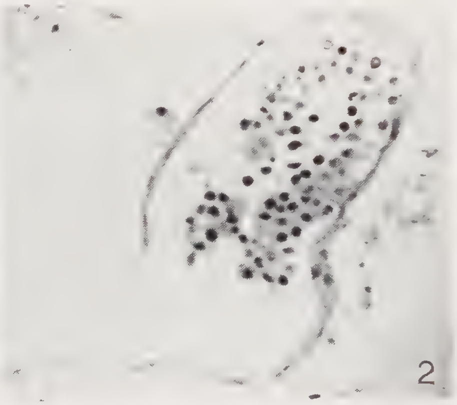

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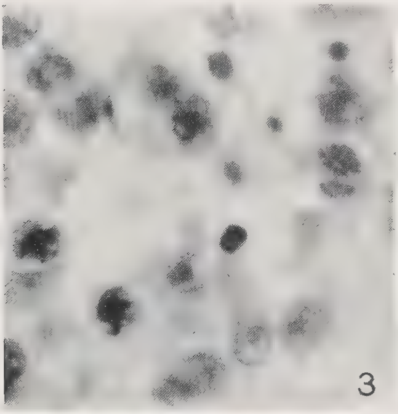
The tissue was fixed in formalin and stained with Delafield's hematoxylin and eosin. Magnifications stated are before reduction. Figures are reproduced at one-sixth off.

Fig. 1 Low power view of tubule in situ. Microscopic examination of the section itself shows the connective tissue continuous with the wall of the testicular tubule and the capsule of the suprarenal gland. Other sections show continuity between the tubule and the vessels which appear at lower left. Still other sections show more connective tissue between tubule and capsule.

Fig. 2 Another section. Tubule somewhat compressed at autopsy. × 225.

## DISCUSSION

No reference to similar human cases has been found in the literature. Champy ('31) reported a somewhat similar condition in a fowl. A cock castrated at 3 months and autopsied at the age of 18 months presented a capon type of comb and could crow. At autopsy no residual nodule of testis was found, but the suprarenal was a little large. Histological examination revealed debris of testis and epididymis lying exterior to the capsule at one pole of the gland. The testicular epithelium was partially regenerated, with low epithelium. The epididymis was better developed. At the other pole there were less well-organized tubes inside the cortex, with single gonadal cells mingled with suprarenal cells.



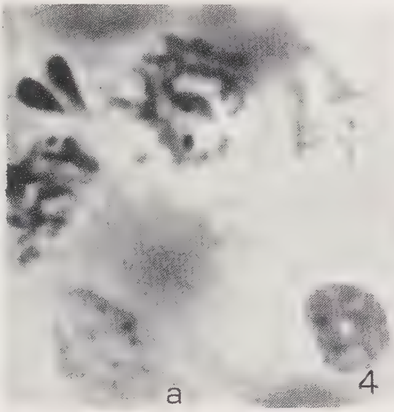


Fig. 3 First spermatocytes in prophase; Sertoli nuclei. × 900.

Fig. 4 First spermatocytes in prophase showing distinct chromosomes; also two sperm heads; Sertoli cell at a. 1.9 oil immersion objective. × 2200.

In some previous castration experiments, Champy had observed enlarged suprarenals, but they were not examined histologically. He also states in a footnote that he had found suprarenal tissue in the testis of a cat. He suggested the advisability of clinical examination of suprarenal tumors for similar phenomena. He also suggested an embryological explanation of his case: the suprarenal gland, which is larger before birth, had engulfed cells of the testicular anlage.

As an explanation of the case reported in this paper, the authors suggest that a mingling of the anlagen of the suprarenal gland and the testis might occur in human embryos of the sixth or seventh week (compare Keibel and Mall, vol. 2, fig. 117).

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# A FACTOR INFLUENCING THE BILATERALITY OF THE EYE RUDIMENT IN HYLA REGILLA

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THREE FIGURES

## INTRODUCTION

Recent studies on experimental cyclopia and on the normal determination of paired eyes have centered themselves on an analysis of the role of the mesodermal substrate. Various experimental procedures show conclusively that the mesoderm underlying the ectodermal eye anlage not only induces the formation of eye tissue, but also is primarily concerned with the formation of paired eyes. The excision of the rostral mesoderm (Mangold, '31; Adelmann, '37) as well as its physiological inhibition by means of lithium chloride and other chemical agents (Lehmann, '33) results in cyclopean embryos. Furthermore, median pieces of anterior neural plate, transplanted without substrate to the flank, form single eyes, but when transplanted with the substrate, form paired eyes (Adelmann, '30; Alderman, '35). It is evident that the normal pairing of the eyes, which originate in a single median dispersion circle, is referable to factors in the mesoderm.

I have demonstrated the lability of the ectodermal eye field in a previous paper (Alderman, '35). In the early neurula stage of Hyla regilla, the rotation of rostral squares of neural plate through 180° results in the development of normal larvae. In such a case, eyes form from tissues which would normally have been brain, and brain tissue is differentiated from the eye forming area. If instead of rotation, the anterior end of the excised piece is transplanted to the flank, it continues along preestablished lines and forms a single eye. Therefore we can conclude that at this time the eye field is in a truly labile state of determination. It is also important to note that the rotation experiment shows a new action of the mesoderm: When the eye forming areas are brought into contact with more posterior mesodermal areas, eye formation is inhibited.

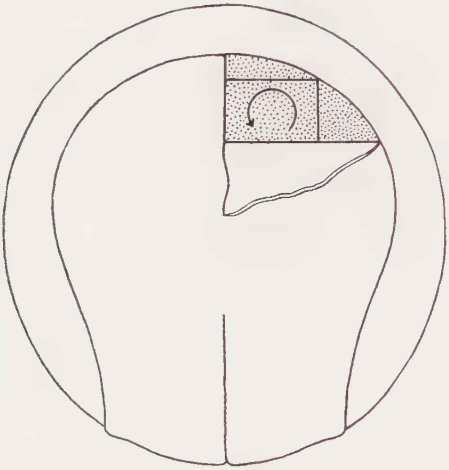

Fig. 1 Diagram showing type of operation performed. The anterolateral square of medullary plate is lifted, and the underiying substrate is rotated 180°. The sector of medullary plate is then replaced in its normal position.

During the course of the experimental work just described, the possibility arose that inhibition of eye tissue formation might be of functional significance in the normal splitting of the eye field, and the consequent formation of paired eyes from a single anlage. This could easily be examined by making known displacements of the mesoderm underlying the eye field. The results should show 1) whether the mesoderm is physiologically bilaterally symmetrical in this stage of development, and 2) whether a median, eye inhibiting area exists which splits the single eye field.

## EXPERIMENTS

The experiments designed to answer the above questions were performed as follows: The anterior end of the neural plate of the early neurula stage of Hyla regilla was lifted and a lateral square of mesoderm, as shown in the diagram (fig. 1) was rotated 180°. Since neither the existence nor the boundaries of an eye inducing dispersion field in the mesoderm were certain, arbitrary boundaries for the rotated square were chosen. In all cases the medial edge of the square corresponded as closely as possible with the midline of the embryo, and the lateral edge was halfway between the midline and a line continued anteriorly from the edge of the neural plate at its widest point. Thus if an eye inhibiting area exists in the midline, and an eye supporting area exists laterally, their positions are reversed on the operated side, and normal on the unoperated side.

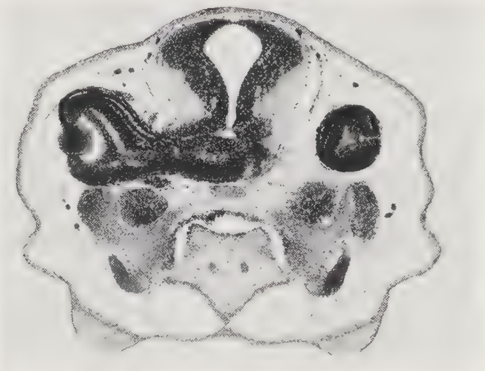

Fig. 2 Experiment E-101. Cross section through eye region. Eye on operated side fused to diencephaon. Unoperated side normal, although reduced in size.

A preliminary control series was made, in which the mesoderm was lifted out and replaced in its normal orientation. The neural plate was then replaced. All of ten embryos in this series were normal. It was concluded that the operative effects were of no significance in the results. There was no mortality throughout the experiments.

## RESULTS

Of thirty-one experimental embryos in this series, all but one showed asymmetrical development of the eyes. Seventeen of the embryos showed an abnormal or reduced eye fused to the diencephalon on the operated side, while the eye on the non-operated side was normal or reduced in size (fig. 2). It will be noticed in the figure that eye tissue on the operated side continues medially to the diencephalon. There is no optic nerve.

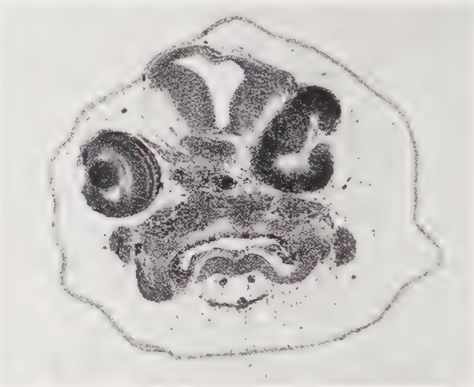

Fig. 3 Experiment F-23. Cross section through eye region. Eye on operated side doubled, and pressed closely against diencephalon. Unoperated side normal.

Certain other asymmetrical conditions, grading into the condition described above, were observed. Doubled eyes appeared in five cases on the operated side. In all cases they were closely applied to the wall of the diencephalon (fig. 3). In five cases the eye on the operated side was normal or reduced in size, but was also closely pressed against the brain wall. In three cases the eye on the operated side was completely absent.

A recapitulation of the results appears in the following table:

*Table showing distribution of results after rotation of rostrolateral mesoderm 180°*

| *Operated side* | *Unoperated side* | *Number of embryos* |
|---|---|---|
| Fused to diencephalon | Normal | 17 |
| Doubled and applied to diencephalon | Normal | 5 |
| Normal or reduced and applied to diencephalon | Normal | 5 |
| Absent | Normal | 1 |
| Absent | Fused to diencephalon | 2 |
| Normal | Normal | 1 |

## DISCUSSION

It is evident from the results that the potentialities of the anterior mesoderm underlying the midline are different from those of more lateral regions. In the majority of cases the eye on the operated side was fused to the diencephalon. In such cases it seems logical to conclude that the eye supporting mesoderm has been shifted medially by the rotation, and has come to lie under the region of the neural plate which normally gives rise to optic stalk. Thus, instead of eye suppressing mesoderm, there is now eye supporting mesoderm under the eye field in this region and consequently eye tissue has been formed. Furthermore it will be noted that in all but four cases the eye has come to lie closer to the diencephalon than the eye on the unoperated side. It must therefore have formed from more medial tissue of the neural plate. It is a well-known fact (Adelmann, '30; Alderman, '35) that the tissue of the plate from which eyes normally form possesses at this time certain potentialities for eye formation, since if it is transplanted to a relatively neutral environment, such as the flank, it differentiates into eye tissue. We must conclude, then, that since eye tissue has not formed in its normal position, these potentialities must have been inhibited by the median mesoderm which has come to underlie a part or all of the normal eye forming region.

The two cases in which the eye on the operated side was completely absent, and that on the non-operated side was fused to the diencephalon, are particularly interesting. The protocol shows that the median cut was somewhat over the midline. In such a case the eye supporting mesoderm would have been shifted to the opposite side, so as to approximate the contralateral eye supporting mesoderm. As a result, the eye on the operated side is completely inhibited by the action of all of the median mesoderm, and the eye on the unoperated side is fused to the diencephalon. Further experiments are planned in order to ascertain whether or not this explanation is tenable.

## SUMMARY AND CONCLUSIONS

1. Evidence is given in support of the view that the mesoderm underlying the eye field constitutes a mosaic in the early neurula.

2. Two eye supporting mesodermal areas induce the formation of paired eyes from the harmonic equipotential eye field.

3. The material between the eye supporting regions has a different action, inhibiting rather than supporting eye formation. It seems probable that the single dispersion circle is split during development into two bilaterally arranged eye fields by a median suppression of eye formation.

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# INCREASED SENSITIVITY OF THE VAGINA TO THEELIN, IN THE CHRONIC CASTRATED RAT, AS INDUCED BY THE COTTON SWAB PROCEDURE

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ONE TEXT FIGURE AND ONE PLATE (FOUR FIGURES)

The vaginal epithelium of the rat is very sensitive to a cotton swab and false readings of estrus are sure to follow when frequent vaginal smears are made in this way. The pipette method, on the other hand, seldom causes cornified cells to appear in the smear (Emery and Schwabe, '36). The effect of the cotton swab seems all out of proportion to the irritation produced, especially so in these experiments, since the swabs were wet with physiological salt solution. In addition, we failed to get anything like the cotton effect when a rough wire spatula was inserted into the vaginal canal and given about the same amount of twisting as was used on the handle of the cotton swab. In the present report we have studied the after effect of the cotton swab as tested by the responsiveness of chronic castrated rats to theelin.

## PROCEDURE AND METHODS

The rats used were oophorectomized at or just before sexual maturity (in some cases the vagina was open). Six to 10 months later they were divided as to weight and general physical condition into several groups. Five to ten rats were placed in each group and two groups were started each week. This gave us more samples should error occur in the procedure. The usual procedure was to start one group on theelin and the other on cotton swabs for 3 days. Five days later this procedure was reversed, theelin now being given to the group previously on the cotton swabs, while those previously injected with theelin were now examined with the cotton swab. The theelin was given subcutaneously in 1 R.U. doses twice daily for 3 days, total of 6 R.U. The vaginal smears in the theelin groups were always taken twice daily by the pipette method, i.e., using a medicine dropper and normal saline solution. The cotton swab smears were taken four times daily for 3 days, followed by two smears daily with the pipette method until all rats were in diestrus. In a few experiments the usual 3-day treatment with the cotton swab was extended to 5 days in order to give more pronounced effects. The data from these experiments we have placed in figure 1.

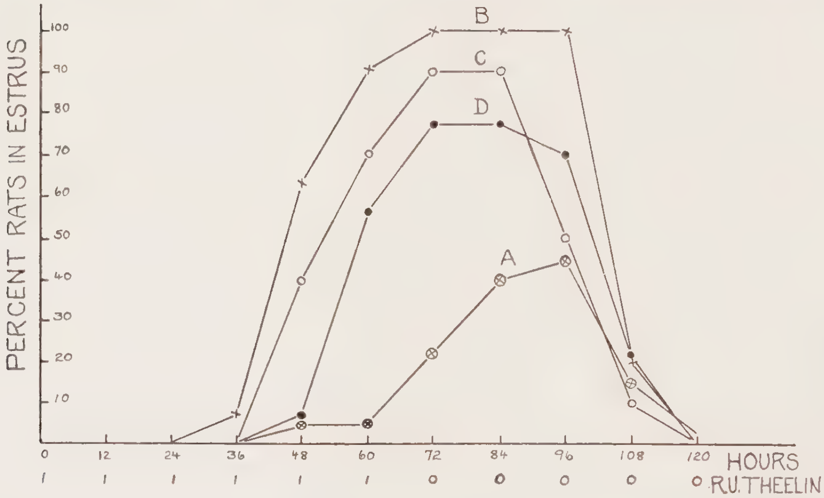

Fig. 1 The per cent of rats in estrus each 12 hours for a period of 5 days, together with the rat units of theelin injected. A, forty rats with no previous treatment. B, fifteen rats—the vagina was swabbed with cotton four times daily for a period of 5 days. This was followed by a rest period of 10 days, then theelin injected as shown. C, ten rats—same as group B but followed by a rest period of 20 days. D) fifteen rats—same as groups B and C but followed by a rest period of 30 days. All rats oophorectomized at 40 days of age and 6 to 10 months before the experiment started.

## RESULTS

The cotton swab method of vaginal examination produced an estrous-like condition in these chronic castrated rats. Four smears daily over a period of 3 days induced positive vaginal smears in 40% of these animals at the end of 72 hours (table 1 group E). This result might be expected since similar changes were known to occur in rats more recently castrated (Wade and Doisy, '35; Emery and Schwabe, '35, '36; and Rogers and Allen, '37). Interest in these false estrous-like periods is further aroused by the fact that the uterus and vagina of these animals were in a very chronic castrated state. This is further emphasized in group F, table 1, where 6 R.U. of theelin produced estrus in only about half of the rats. The estrus produced by theelin is probably a manifestation of a more complete stimulation to the entire genital tract than can be obtained by the estrous-like condition produced by the cotton swab. Yet, as we previously pointed out (Emery and Schwabe, '36), the cornified smear produced by the cotton swab procedure cannot be distinguished from that taken from a normal rat in estrus. Besides, the effects of cotton swabbing seem long-lasting—as discussed below.

TABLE 1

*The per cent of rats in estrus for each 12-hour period up to 120 hours is shown. Cotton swabs given four times daily for 3 days. Theelin given in doses of 1 R.U. twice daily for 3 days. In each group 5 days after the first treatment the second treatment was started. All rats oophorectomized before sexual maturity and 6 to 10 months before treatment*

| GROUP | TREATMENT | NUMBER OF RATS | HOURS AND PER CENT IN ESTRUS | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | 12 | 24 | 36 | 48 | 60 | 72 | 84 | 96 | 108 | 120 |
| A | Theelin | 40 | 0 | 0 | 0 | 0 | 5 | 5 | 22 | 42 | 45 | 15 | 2.5 |
| | Cotton | 40 | 0 | 2.5 | 25 | 40 | 65 | 80 | 75 | 20 | 5 | 0 | 0 |
| E | Cotton | 40 | 0 | 0 | 5 | 7.5 | 30 | 35 | 40 | 2.5 | 5 | 0 | 0 |
| | Theelin | 40 | 0 | 0 | 9 | 35 | 52 | 75 | 85 | 87 | 57 | 15 | 2.5 |
| F | Theelin | 20 | 0 | 0 | 0 | 0 | 10 | 10 | 20 | 45 | 55 | 20 | 0 |
| | Theelin | 20 | 0 | 0 | 5 | 25 | 35 | 30 | 25 | 60 | 15 | 5 | 0 |

The duration of the effect of the cotton swabs on the vaginal epithelium seemed at first only temporary since the positive vaginal smears rapidly returned to the diestrous condition when the swabs were discontinued (group E, table 1). However, further study showed that the effects were more prolonged than the vaginal smears indicated. This was evident when these same rats, after a 5-day rest period, were injected with theelin. They responded quickly; estrus began to occur earlier than in the controls and a larger per cent of the rats were in estrus each day. The figures in the latter case reached a maximum of 87% (group E) as compared to 45% and 55% obtained in the control groups A and F in which the first treatment was theelin. Thus, in these experiments, cotton swabbing seems to sensitize the vaginal epithelium to theelin. This effect is greater than that induced by a previous treatment of 6 R.U. of theelin and is shown by comparing the data given for the second treatment in groups E and F, table 1. Following cotton swabbing, 6 R.U. of theelin induced estrus in 87% of the rats at the 84-hour period, while only 60% of those which had previously been treated with theelin were in estrus. In contrast to this, it is interesting to note that the duration of the estrus induced by theelin lasts the same length of time in all groups so that by 120 hours practically all of the rats were again in diestrus (table 1).

The theelin overcame some of the atrophy of the vagina in these rats so that, after a 5-day rest period, they were more responsive to a second series of theelin injections (group F, table 1). Likewise, a previous treatment with theelin made the vagina more responsive to the cotton swab method of inducing estrus. This is shown by comparing group A with group E, table 1. In group E the maximum number of rats showing positive smears at one time was 40%; while in group A, where the theelin was first given and after 5 days followed by cotton swabs, this figure rose to 80%.

The effect of cotton swabs on the sensitivity of the vagina to theelin is not only in evidence after 5 days, as mentioned above, but may in fact last for more than a month. The data on this point we worked out again by following the cotton swabs with theelin. After intervals of 10, 20, 30 and 40 days these rats were given the usual dose of theelin used throughout these experiments, namely, 1 R.U. subcutaneously twice daily for 3 days. In order to make the effects more pronounced, a 5-day period for application of the cotton swabs was chosen instead of the usual 3-day period just described.

The results were very gratifying, for not only did the vaginal epithelium become responsive to theelin comparable to the reaction seen in more recently castrated rats, but also, this sensitizing effect was still evident to a limited degree even after a rest period of 40 days. The data obtained at 40 days were not numerous enough to analyze further, but the other data can be seen in figure 1. Curve A represents the forty rats shown in group A, table 1. Curves B, C and D show the per cent of rats in estrus induced by the same dosage of theelin started 10, 20 and 30 days respectively after the cotton swabs were discontinued. During these rest intervals no theelin was given and no vaginal examinations were made; thus, the effect noted represents the residuum of the sensitivity induced by the cotton swabs previously given.

## DISCUSSION

The fact that the application of cotton swabs increases the sensitivity of the vaginal epithelium to theelin makes this method of obtaining vaginal smears very unsatisfactory. Certainly a suitable rest period of several weeks duration should follow the application of the swabs before the response to theelin is comparable to that obtained in castrated rats of long standing. In our experience the cotton swab method will always give more positive readings than the pipette method. This is especially true of rats either coming into or going out of estrus where the smear is slightly positive when taken by the pipette method; in such cases if the rats are immediately examined by the cotton swab procedure the smear becomes positive and scales of degree +++ or ++++ are frequently seen. This confirms our previous report ('36) in which the advantages of the pipette method were discussed.

In order to study further the factors concerned in the sensitization of the vaginal epithelium some of these rats were sacrificed for histological material. Sections of the uterus show this organ to be unaffected by the cotton swabs. On the other hand, the vagina in the rats swabbed with cotton showed the epithelium to be in active proliferation, the number of cell layers depending upon the amount of desquamation. The submucosa shows edema, hyperplasia and extensive leucocytic infiltration (fig. 3). After a 10-day rest period the mucosa has practically returned to normal but the submucosa is still somewhat edematous and hyperplastic (fig. 4). This latter condition may be in some way connected with the sensitivity of these rats to theelin.

The theelin [1] solution made fresh daily in a concentration of 1 R.U. per cubic centimeter, was kept in the ice box. All rats received the same amount twice daily at the same time each day. This precaution is probably unwarranted, yet, judging from the most recent report on dosage of theelin, one must still be cautious if one wishes to speak in terms of units (D'Amour and D'Amour, '38).

## SUMMARY

1. The chronic castrated rat 6 to 10 months after oophorectomy is capable of showing positive vaginal smears when examined four times daily with a cotton swab.

2. Rats that have been examined with the cotton swab become more sensitive to theelin than the controls. This effect may still persist after a rest period of several weeks.

3. Cotton swabs were more effective than small doses of theelin in sensitizing the vaginal epithelium as judged by subsequent reactions to theelin in these rats.

4. The disadvantage of the cotton swab method of vaginal examination is again pointed out.

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[1] We are grateful to Dr. Oliver Kamm, Parke, Davis and Company for supplying us with theelin.

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# PLATE 1

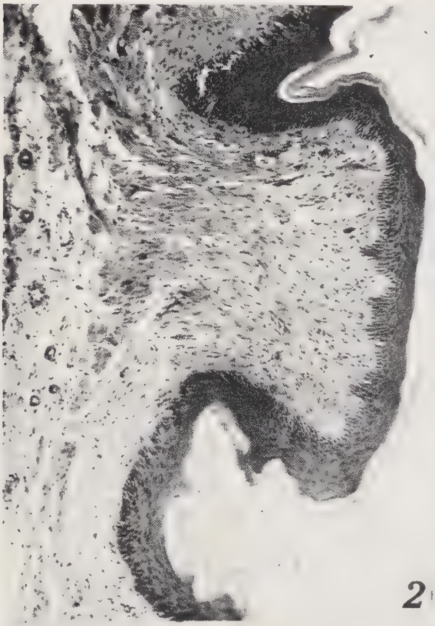
## EXPLANATION OF FIGURES

2 Cross section of vagina of a normal rat in estrus. × 75.

3 Cross section of vagina of a chronic castrated rat after a 5-day period during which a cotton swab was applied four times daily. × 75.

4 Rat subjected to same treatment as animal in figure 3 but killed after a 10-day rest period. Note the extensive leucocytic infiltration, and lack of cornified cells. × 75.

5 Cross section of vagina of a rat 6 months after an oophorectomy performed at 40 days of age. × 75.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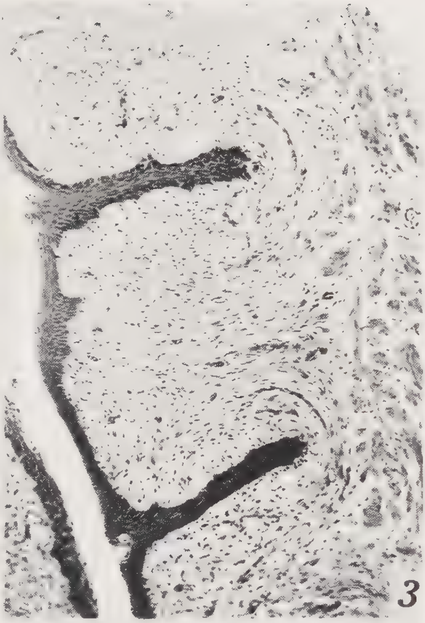
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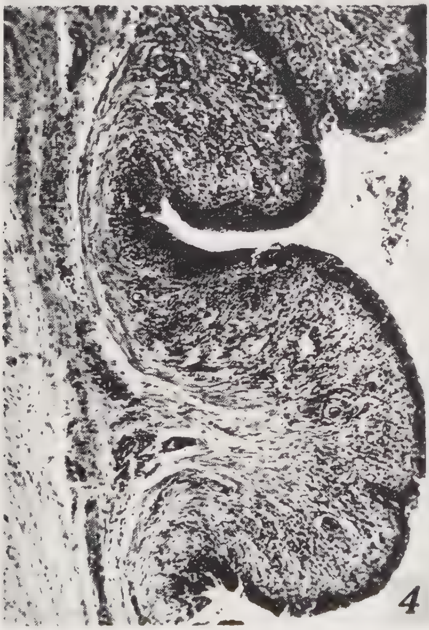
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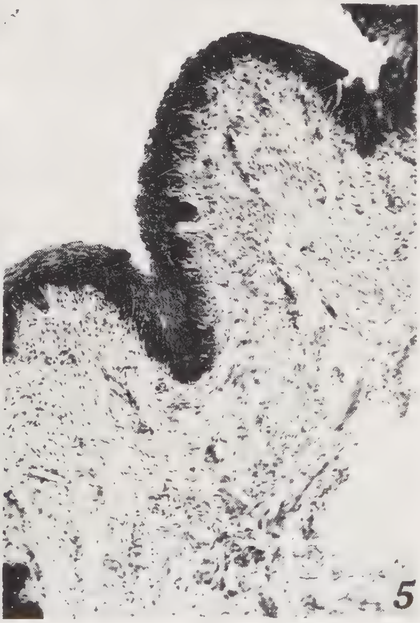
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# THE ANATOMY AND DEVELOPMENT OF THE SACRO-ILIAC JOINT IN MAN

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ELEVEN FIGURES

Although this investigation is chiefly concerned with the development of the sacro-iliac articulation, it was thought desirable to include a few observations on its gross anatomy. It is regrettable that an adequate idea cannot be obtained from any standard textbooks of anatomy in English. The descriptions are fragmentary, conflicting and misleading. Little mention is made, for example, of the considerable variability in size, shape, and contour of the auricular surfaces, giving the impression that they all conform to the same pattern. Yet they vary not only from individual to individual but also from side to side of the same body.

The shape of the auricular surfaces is usually that of an L with the long arm directed dorsocaudally, and the short arm dorsocranially, generally at right angles to the long one. Sometimes the two arms are of nearly equal length, resembling the letter V, with varying angles. However, the short arm may be absent, the auricular surface forming a rectangle, or a triangle, and intermediate types also occur.

I found the diagonal length of the auricular surfaces in different adults to vary from 5.3 cm. to 8 cm., and the width from 1.8 to 4.1 cm. As usually stated, the auricular surfaces on the sacrum included the first two sacral vertebrae and very often a part of the third. These surfaces probably remain flat until some time after puberty, as is generally believed, for those from a 13-year-old female still were in this condition and smooth and glistening with only fluid enough to moisten the surfaces. In the adult, however, plane surfaces are the exception, for a longitudinal iliac ridge which fits into a sacral groove frequently is present. The reverse condition is sometimes present, and almost every conceivable combination of grooves, ridges, eminences, and depressions may occur. Eminences are commoner on the ilium, though frequently present on the auricular surface of the sacrum.

The sacral articular cartilage of the specimens examined varied from 1 to 3 mm. in thickness, while the iliac cartilage usually was less than 1 mm. thick. The former was hyaline for the most part with surface cells arranged in compact, parallel layers, and with a more fibrous matrix. In adult joints it often seemed as though superficial degeneration had occurred, for only fibers remained. The presence of islands of hyaline cartilage between columns of fibrocartilage and set perpendicularly to the articular surface, gave a characteristic appearance to the iliac articular cartilage. The compacta on the auricular surface of each side had many small perforations permitting marrow and blood vessels to come into contact with the cartilage.

After the third decade the surfaces of the apposed cartilages were roughened, furred, and frayed, phenomena which have been attributed to a fibrous transformation by Sashin ('30). However, microscopic examination of the larger frayed tags revealed that they, like the interauricular bands to be described later, are not composed of fibrous tissue. They resembled ragged, worn, degenerate areas found in other articular cartilages with normal cartilage intervening between their fixed ends and the bone. The significance of this change remains unknown to me. That it may be the result of wear, such as described in other joints by Meyer ('24), is possible. However, in that case it would seem that one should occasionally find the cartilages completely worn away in some places leaving eburnated bony areas so commonly present in other joints. In saying this I do not imply, however, that motion is entirely precluded at the sacro-iliac joint, though I was unable to find any polished facets in the cleaned bones from over 200 adults.

The freshly exposed auricular surfaces were always moist in dissecting room cadavers and in 2 to 3%, a small amount of fluid (1 or 2 drops) appeared on the surface when the articular capsule was penetrated ventrally by the point of a knife. This fluid was usually a little denser than synovia, but lacked its characteristic slipperiness and was more abundant in females.

The dorsal non-auricular articular portions of this joint also present interesting gross variations. Occasionally they are flat and lie sufficiently in one plane so that the sacro-iliac ligaments can be divided with in thin knife from the dorsal aspect. Oftener, however, they are quite curved and irregular, with eminences and depressions so closely approximated that it is impossible to cut these interosseous ligaments without cutting through portions of the bones. Frequently there is a wide and deep fossa in the ala of the sacrum just dorsal to the auricular surface, and although there usually is a corresponding eminence or boss on the ilium, it rarely is large enough to fill completely the coapted sacral fossa.

The frequent occurrence of supernumerary articular facets dorsal to the auricular surfaces is not generally recognized, yet one or more were detected in sixteen of forty-seven pelves examined for this purpose. As many as three were found in one specimen. They varied in diameter from less than 2 mm. to over 1 cm. Microscopic sections through one of these supernumerary articulations showed both surfaces to be composed of fibrocartilage. Further information regarding them in cleaned pelves, can be found in the careful and comprehensive study by Trotter ('37).

Motion in the sacro-iliac articulation has been demonstrated by skiagrams because rotation at the sacro-iliac joint causes forward displacement of the pubic bones at the symphysis, on the side upon which the individual is bearing his weight in standing. In joints with a flat auricular surface there is nothing to prevent motion except the sacro-iliac ligaments, but since most sacro-iliac joints have irregular, interlocking auricular surfaces and strong, relatively rigid interosseous ligaments, it is difficult to believe that more than the very slightest amount of motion is possible in these persons, for when all ligaments surrounding this joint have been cut and the auricular surfaces are pressed firmly together manually, practically all motion is prevented by the interlocking surfaces. The little motion that can be effected in some specimens is rotation on a transverse axis, but gliding or slipping sufficient to produce anything resembling a subluxation seems impossible.

It has often been said that the alleged reduction of a certain type of sacro-iliac dislocation by a 'thrust' sometimes is accompanied by an audible 'click.' To dislocate a fair-sized eminence from its apposed depression would require a considerable amplitude of motion, but since supernumerary sacro-iliac facets sometimes merely are tiny elevations or tubercles, only slight motion would be required to dislocate them or other slight irregularities upon the auricular surfaces. Tension of the interosseous ligaments would tend to keep such prominences dislocated and resist the separation necessary for their relocation, though they might be made to 'snap' back into position by more or less strenuous manipulation.

The use of the term 'anterior sacro-iliac ligament' to include fibers over the inferior portion of the anterior margin of the joint seldom seems justified for a definite ligament is present superiorly only. Inferiorly the bones are united only by joint capsule, although in bodies of middle age and over, there is frequently a thickening of the capsule anteriorly, especially in its superior portion. There may also be a fibrocartilaginous ridge over the anterior margin of the joint consisting mostly of hyalinized connective tissue, but containing a small amount of fibrocartilage. This ridge is a helpful landmark in opening the joint. Moreover, partial bony ankylosis between the upper ventral portions of the ilium and sacrum, which is common after the fifth decade especially in males, is usually preceded by exostotic reaction about the joint margins. It nearly always begins at the dorsal extremity of the anterior-superior portion of the capsule which becomes transformed into a bridge of bone as does the anterior sacro-iliac ligament just dorsal and cranial to it. As this bony bridge widens progressively, mainly downward, it also becomes thicker and ossification may even invade upon the joint cavity. Extra-articular ankylosis existed in nine out of twenty-one adult bodies. In seven of these it was bilateral and it occurred on the right side in six, which suggests that function may be a factor in its production. Moreover, when bilateral, it was usually more extensive on the right side. In one joint bony ankylosis was continued to the region of interosseous ligaments. Indications of inflammatory processes were absent and the process did not seem directly related to senility, for the average age of the bodies showing bony anklosis was considerably less than those without it. However, a much larger series must be studied before final conclusions are justified.

The embryology of joints belongs to a rather neglected field of recent investigation. This is particularly true of the sacro-iliac joint. Because of the lack of knowledge upon the development of this joint, frequent clinical involvement of one or both sacro-iliac joints, and the interesting structural changes so often seen in later adult life, further study seemed desirable. Much has been written concerning the structure, function, pathology, and surgery of the sacro-iliac joint, but little has been done on its development. Moreover, a considerable search of the literature did not reveal adequate information and the statements in the textbooks are conflicting.

Pilatte and Vignes ('19) stated that, "The cavity appears during the tenth week by a cleavage in the mesenchymatous mass which adjoins the chondrogenous zones. By the fourth month the articulation has its definite constitution, although the articular surfaces at this period are flat and conform more to an arthrodial type than to the complex type of the adult.'' In regard to bands connecting the apposed developing articular surfaces in the infant, they said that, ''In the infant, the articular surfaces are frequently united by fibrocartilaginous bands which bear witness to a retardation in the development of the articular cavity,'' but I was unable to find any report of the investigations on which these statements apparently were based.

The occurrence of such bands was questioned by Brooke ('23), who said that, ''In younger subjects of both sexes the joint surfaces are quite smooth, and separate, and the presence of congenital fibrous strands bridging across the joint described by Henry Vignes must be an extremely rare condition.'' However, Prentiss ('17), in commenting on his figure 2, a drawing of the sacro-iliac joint of a newborn infant, wrote, ''Here we observe a distinct cavity lined by a modified fibrocartilage on both opposing surfaces, and not by hyaline articular cartilage. A few fasciculi are observed connecting the two opposed surfaces, indicating an effort at the persistence of the developmental condition.''

Although my investigation revealed when and where the cavity begins, the process of cavitation remains obscure. A review of work on this subject prior to 1902 is contained in the paper of Retterer ('02), who from his own work on sheep, horses, rabbits, and guinea pigs concluded as follows: ''At the point of meeting of the skeletal segments the skeletogenic tissue undergoes either a mucous, reticular-connective, or fibrous transformation. In the region of the future interline the reticular connective tissue becomes mucous, then liquefies; around the articular cleft the reticular tissue becomes vascularized and persists as synovial membrane.'' It is noteworthy that Retterer found ''red blood cells and leukocytes'' present in the early articular cavities.

Thirty-five formalin-preserved human specimens varying in vertex-breech length from 25 mm. to 46.5 cm., and in age, according to Streeter's ('20) graphs, from 8 weeks after conception to 9 months post natum, were examined. In fetuses over 8 cm. (V.B.), the lower extremities were amputated at the thighs and the trunk transected between the fourth and fifth lumbar vertebrae. The soft tissues, except the ligaments, were then removed to expose the pelvis. The symphyseal region was also excised and one sacro-iliac joint, alternately the right or left, was opened for gross examination by cutting the anterior portion of its capsule. The other joint was then removed, unopened, for microscopic examination of serial sections. Pelves of specimens under 8 cm. were sectioned serially in toto, into 15 μ sections, frontally and transversely. The number of sections studied varied from complete serial sections in the younger specimens to three per millimeter in the older ones. Some sections were stained with Delafeld's hematoxyylin and counterstained with 1% aqueous eosin. Others were stained with Hansen's hematoxylin and counterstained with acid picrofuchsin.

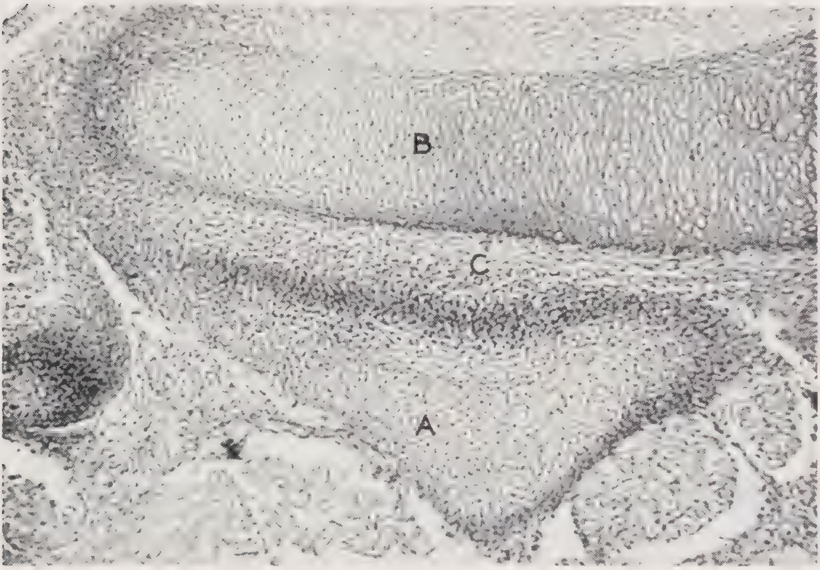


Fig. 1 Photograph of a transverse section through the midportion of the left sacro-iliac joint of a 25-mm. (V.B.) fetus (8 weeks); A, sacral cartilage; B, iliac cartilage; C, interposed zone of mesenchyme. × 100.

It was unnecessary to use fetuses smaller than 25 mm. (8 weeks), for at this period of development (fig. 1) cavitation had not yet begun, although the sacral and iliac cartilages were well differentiated. Each was covered by a zone of compact mesenchyme, thicker around the sacrum and most marked in the region of the future articular cavity, with the two cartilages separated by a mass of mesenchymal cells which were more loosely arranged and had paler staining nuclei. This mass of mesenchyme had approximately one-third the thickness of the adjoining iliac cartilage but rapidly became thinner as the sacral and iliac cartilages grew and approached each other.

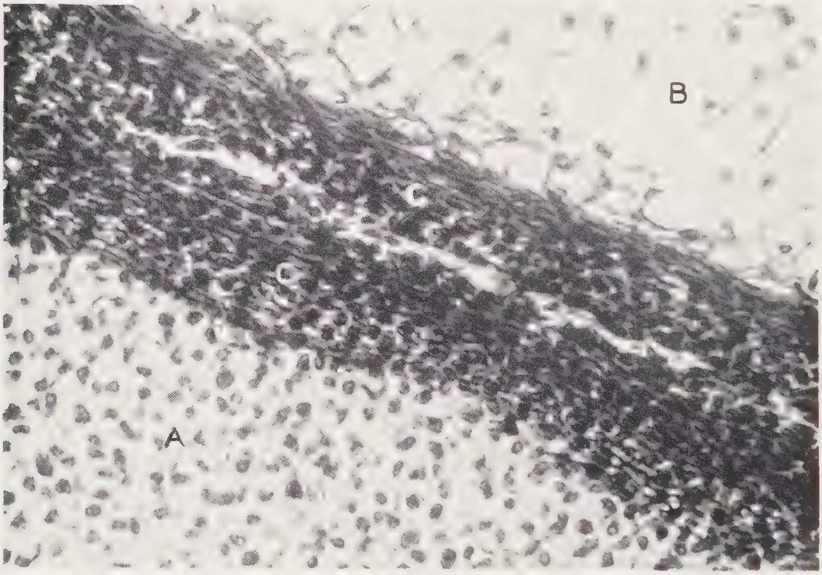


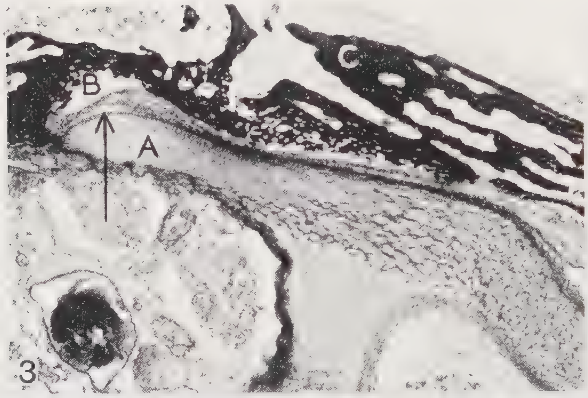
Fig. 2 Photograph of a transverse section through the midportion of the right sacro-iliac joint of a 40-mm. (V.B.) fetus (approximately 10 weeks). A, sacral cartilage; B, iliac cartilage; C, interposed zone of mesenchyme containing a slit which is the early articular cavity. × 400.

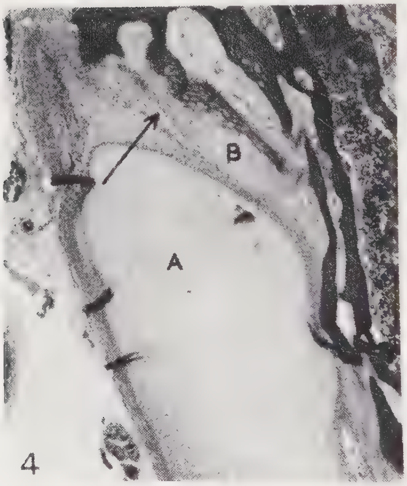
Unmistakable cavitation was first observed in a 40-mm. (10 weeks) fetus (fig. 2). The cartilages at this stage, and for 1½ weeks preceding it, were separated only by their surrounding capsules of dense mesenchyme. In the middle of this interposed zona, and occupying its middle third ventrodorsally, a very narrow and slightly irregular cleft appeared No degeneration was present in the mesenchyme to account for its presence, nor was there any reticular or mucous transformation as described by Retterer in other mammals.

Cavitation proceeded very slowly during the third month. A 7.5-cm. fetus (3¼ months) showed only a very small cavity (fig. 3) which was located in the ventral portion of the joint. This is in accord with the statement of Bardeen ('10) that the formation of the joint cavity "begins at the side and extends toward the center" of all diarthroses, but this is not always true of the sacro-iliac joint, for cavitation frequently begins simultaneously in several places, peripherally and centrally. At this stage of development the adjacent portion of the ala of the ilium had become ossified, but a very thin layer of fibrocartilage remained along the articular surface except upon its ventral portion where there was a thick, columnar mass of fibrocartilage filling a trough-like depression in the iliac bone. The lateral part of the sacrum, included in the section, was represented by a highly cellular fibrocartilage. However, in most specimens it was difficult to tell, even at term, whether the cartilage was fibrous or hyaline. Occasionally it was clearly one or the other, but more often it was mixed. This was also true of the iliac cartilage during the first 4 months, after which it nearly always was fibrous.

As shown in figures 4 and 5, which are of sections from an 11.7-cm. fetus (4 months), the anterior portion of iliac cartilage which forms a separate column was thickest at the caudal extremity of the joint and became thinner as the cranial extremity was reached. It persisted during the third, fourth and fifth months after which it became progressively thinner. The articular cavity was still very small at this time and was found only in the cranial portion of the joint.

The section from a 16.5-cm. fetus (5 months) shown in figure 6 was unique in my series because there was an abrupt transition from fibrous to hyaline cartilage in the sacrum





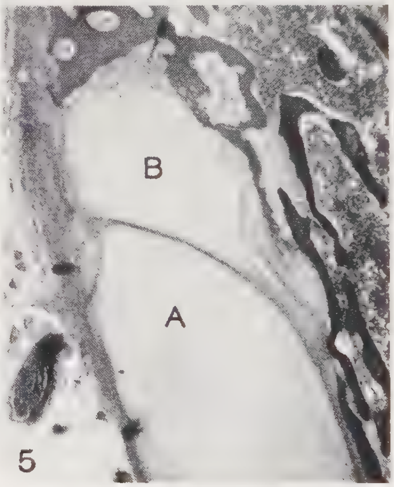


Fig. 3 Photograph of a transverse section through the caudal portion of a left sacro-iliac joint of a 7.5-cm. (V.B.) fetus (3¼ months): A, sacral cartilage; B, iliac articular cartilage; C, iliac bone; the arrow points to the articular cavity. × 30.

Fig. 4 Photograph of a transverse section through the cranial portion of a right sacro-iliac joint of an 11-7-cm. (V.B.) fetus (4 months): A, sacral cartilage; B, iliac articular cartilage; the arrow points to the articular cavity. × 33 enlarged twice.

Fig. 5 Photograph of a transverse section through the midportion of the same fetus, to which cavitation had not yet extended: A, sacral cartilage; B, iliac cartilage. × 33.

about $\frac{1}{3}$ mm. from the articular surface, but it was the only specimen in which this was true. Several cavities were present, the largest one being at the cranial end of the figure though actually in the midportion of the anterior part of the joint. Another large one was present at the cranial extremity of this section.

The dense mesenchymal cells which covered the very young cartilages forming a perichondrium, were now differentiated and had a clearly fibrous structure. It was in this fibrous tissue that the cavities formed, though not all in the same plane. Some lay nearer to one cartilage and some to the other, while still others lay halfway between. As the cavities enlarged, they often overlapped each other though separated by fibrous septa which were short and thick at first, as seen in figures 6 and 8, but later became longer and thinner and persisted until sometime after birth.

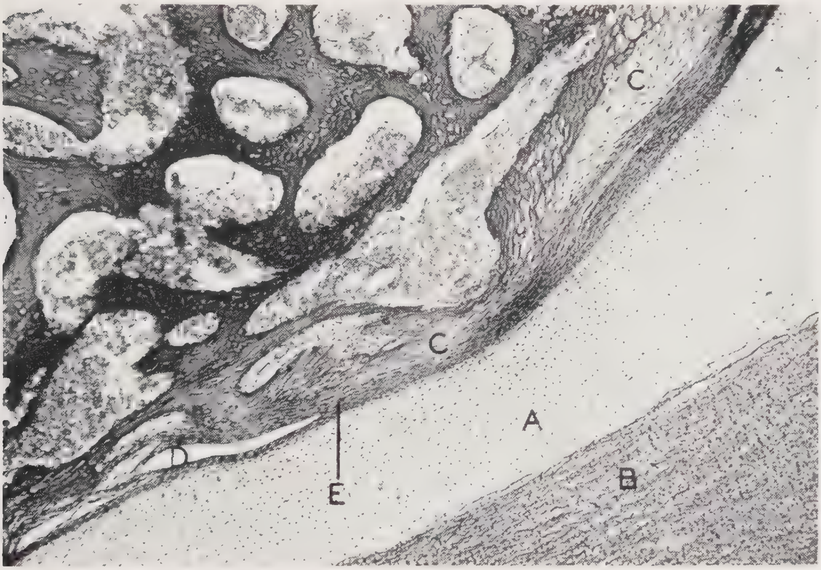


Fig. 6 Photograph of a frontal section through the anterior portion of the left sacro-iliac joint of an 16.5-cm. (V.B.) fetus (5 months plus): A, hyaline sacral cartilage; B, fibrous sacral cartilage; C, iliac articular cartilage; B, portion of the discontinuous articular cavity; E, interauricular septum. × 70.

A transverse section through the middle of the right sacro-iliac joint of a 17.5-cm. fetus (5¼ months) shown in figure 7, contained a thick, fibrous septum bridging the middle of the joint, many thinner ones ventrally and a long one dorsally, the last of which had a thickness of only one cell. Some were rotated accidentally so as to present their flat surface. Hence their true thickness could only be seen at their place of attachment to the articular surfaces.

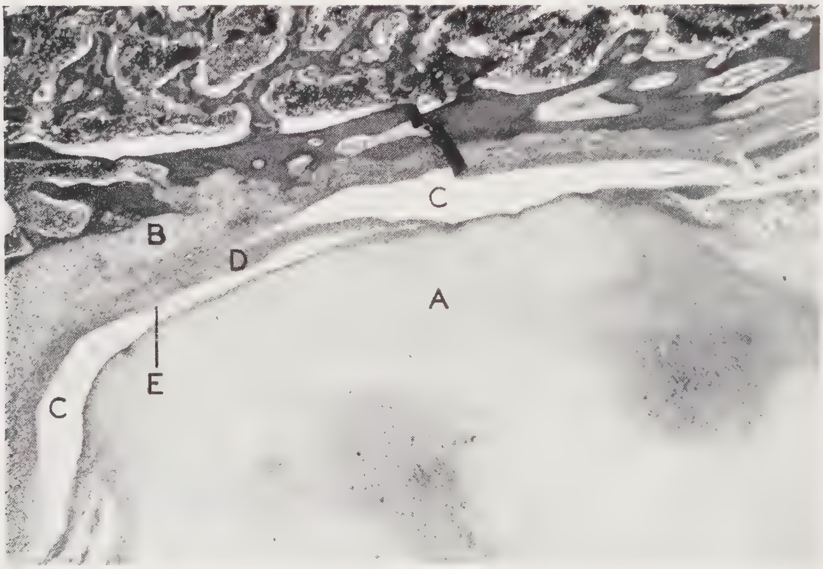


Fig. 7 Photograph of a transverse section through the midportion of the right sacro-iliac joint of a 17.5-cm. (V.B.) fetus (5¼ months): A, sacral cartilage; B, iliac articular cartilage; C, articular cavity; D, thick, fibrous interauricular septum; E, fine interauricular bands. × 35.

The thicker of these septa could be seen easily with the unaided eye upon opening joints past 5 months prenatum and appeared as fine, diaphanous bands which pealed off from the articular surfaces as the bones were separated and were easily torn if the separation was too wide.

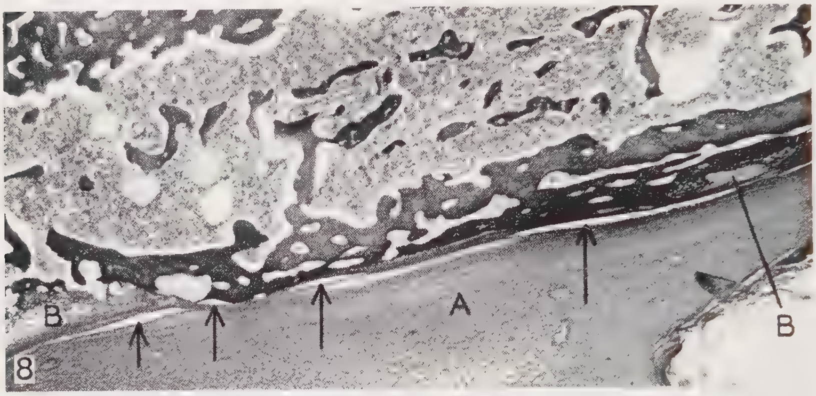
After the sixth month a fine, transverse sulcus usually divided the iliac, auricular cartilage into approximately equal cranial and caudal portions and as the joints were opened most of the delicate bands which remained seemed to radiate from this iliac sulcus to all parts of the sacral auricular cartilage which was devoid of a corresponding ridge. One specimen had several foveolae on the iliac surface with corresponding sacral eminences and in another a transverse iliac ridge fitted into a sacral sulcus. Except for these peculiarities the surfaces were flat, smooth, moist, glistening and without free fluid.

By the seventh month the joints permitted considerable mobility and this mobility increased up to term when the articular capsule and ligaments seemed most relaxed, a phenomenon which may be related to hormonal factors known to affect the sacro-iliac and symphyseal articulations of the mother. At this time the ilium and sacrum may be separated almost 1 mm. without tearing the ligaments. A small amount of movement was possible in any direction, but the joint yielded more readily to rotation on a transverse axis than to any other motion, although motion in the joint became progressively less after birth.

At 6¼ months (fig. 8) the definitive articular cavity was still very incomplete, there being a number of small cavities. The iliac cartilage was thickest caudally and cranially and absent in the midportion of the joint, nothing but a thin, fibrous periosteum intervening between the cavities and the iliac bone.

The joint cavity usually had reached its full relative extent by the beginning of the eighth month, occurring between the lateral portions of the first two sacral vertebrae and the ilium and usually having its characteristic L shape at this time. A section of the joint from an 8-month fetus (fig. 9) shows a complete cavity, bridged by a few thin strands in its midportion only. A transition from a slightly fibrous hyaline to a definite fibrocartilage was present on the sacrum, but the free surfaces of both cartilages were still covered by a fairly thick fibrous layer.

A frontal section through a sacro-iliac joint at term is shown in figure 10. A transverse sulcus was present in the iliac cartilage with a band projecting from its depth. This cartilage was covered by a thick layer of fibrous tissue embedded in a homogeneous matrix, but the sacral cartilage as shown in this section had no such coat perhaps because it was detached in preparation, the strand lying in the middle of the cavity probably being from the sacral side. The sacral cartilage was very vascular, a characteristic of most specimens at or near term.



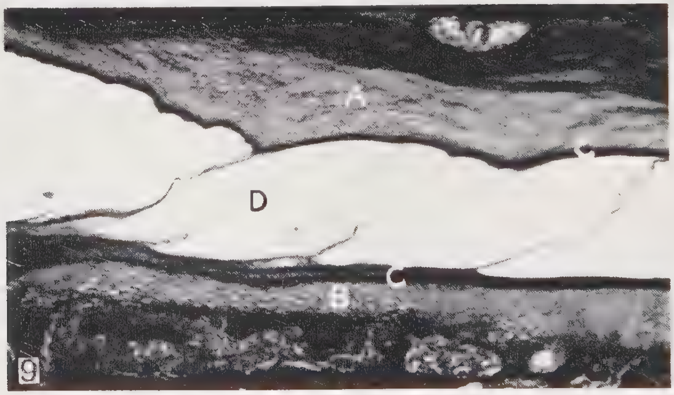


Fig. 8 Photograph of a frontal section through the midportion of a right sacro-iliac joint of a 22-cm. (V.B.) fetus (6¼ months): A, sacral cartilage; B, iliac articular cartilage; arrows point to the discontinuous articular cavity. × 20.

Fig. 9 Photograph of a frontal section through the midportion of the left sacro-iliac joint of a 28.3-cm. (V.B.) fetus (8 months): A, sacral cartilage; B, iliac articular cartilage; C, fibrous surface layer; D, articular cavity crossed by fibrous bands. × 40.

The oldest specimen studied for development was that of an infant of 9 months. On opening the right joint the surface of the iliac cartilage was smooth, but irregular, containing four distinct pits each of which had a corresponding sacral eminence with connecting bands between. In the section from the left joint shown in figure 11 there was a thick, irregular, semi-fibrous bridge connecting the two surfaces. Although the surface layers of each cartilage stained deeply, they had lost their fibrous character, and a layer of flattened cartilage cells in a deep-staining, homogeneous matrix had taken its place. A low eminence was seen on the sacrum at the attachment of the band, and there was a shallow depression on the iliac side. The iliac cartilage was fibrous and the sacral hyaline.

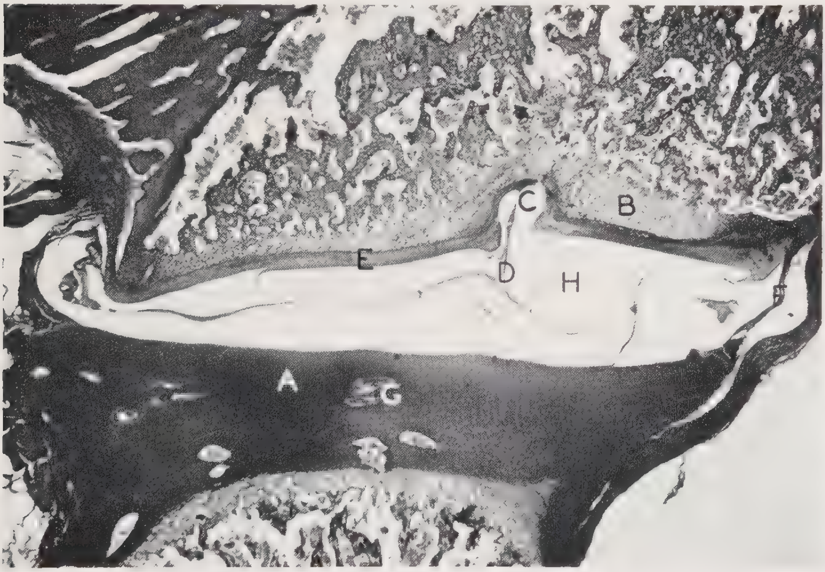


Fig. 10 Photograph of a frontal section through the anterior portion of the left sacro-iliac joint of a 33.7-cm. (V.B.) fetus (approximately term): A, sacral articular cartilage; B, iliac articular cartilage; C, groove in latter; D, intra-articular band; E, fibrous surface layer; F, capsule; G, vascularized channels in sacral cartilage; H, articular cavity. × 14.

A vascular, villus-like projection from the capsule was present in the cranial extremity of the cavity. Under high magnification it was seen that neither this 'villus' nor the capsule was lined by a well-differentiated synovial membrane. Only a few flat surface nuclei were noted, which is in agreement with Sigurdson ('30), who found only 'some differentiation' in the superficial cells of the capsule of the knee joints in 8- and 9-month fetuses. Since the sacro-iliac develops much slower than the knee joint, complete differentiation in it is reached later.

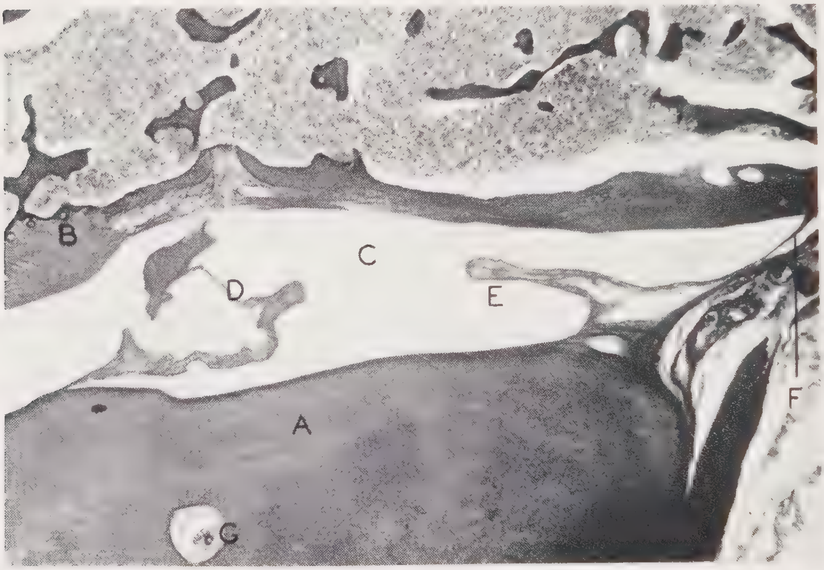


Fig. 11 Photograph of a frontal section through the midportion of the left sacro-iliac joint of a 9-month (approximate) infant: A, sacral articular cartilage; B, iliac articular cartige; C, articular cavity; D, fibrous interauricular band; E, 'synovial villus;' F, capsule; G, blood vessels in sacral cartilage. × 27.

It is regrettable that bodies of older infants and children were not available in order to determine how long the connecting bands in the sacro-iliac joint persist, for interarticular bands are often seen in adult sacro-iliac joints. Microscopic examination of a few has shown them to be composed of neither fibrous tissue nor cartilage but rather of a tissue resembling degenerate, hyalinized fibrocartilage. The bands in adults project from the articular cartilage, which completely covers the bones, and greatly resemble the lining of the iliac cartilage of the term fetus shown in figure 10. That no such bands have been described in these joints of children and young adults may be due to the fact that attention was not directed to them and that few bodies of children are dissected. The presence of normal cartilage between these bands and the respective bones precludes the possibility of their arising from connective tissue of the marrow, as does the fact that they resemble cartilage more than fibrous tissue. Since it is not known that hyaline articular cartilages give rise to fibrous tissue which can grow across articular cavities, it seems probable that the adult interauricular bands have persisted from infancy, but since the adult bands are much larger in caliber than those in the infant they must have grown some while the rest of the joint structures reached maturity. If these bands are new fibrous outgrowths from the marrow through defects in the cartilages, one would have to explain the growing together of the free ends from unapposed areas and uniting with each other in the middle of the cavity.

Red blood cells were occasionally seen in the developing joint cavities, but no correlation could be made between vascularity of the tissue and cavitation. The regions of the interosseous ligaments and capsule were very vascular throughout prenatal development. A few blood vessels also were usually present in the fibrous covering of the articular surfaces, especially adjacent to the capsule and at the base of the larger interauricular bands. In one, a 16.5-cm. (5-month) fetus, relatively large veins were present in the joint cavity.

No fetal sexual differences were detected, but a larger series might reveal some. Although the sacro-iliac joints develop as other diarthrodial joints, cavitation begins later and progresses more slowly.

## CONCLUSIONS

1. In adults the auricular cartilages of the sacro-iliac joints vary greatly in size, shape, and contour, and passive motion usually is very slight.
2. Supernumerary articular facets are common.
3. Roughening and fraying of the auricular cartilages in late adult life is not a fibrous transformation.
4. After the fifth decade partial, extra-articular bony ankylosis is common superio-ventrally, especially on the right side.
5. Cavitation in the sacro-iliac joint begins during the tenth week of intra-uterine life but does not reach its full extent until late in the seventh month.
6. A synovial layer is not recognizable microscopically until sometime after birth.
7. Before birth the sacral articular cartilage may be either hyaline or fibrous but after birth it usually is hyaline. The same is true of the iliac auricular cartilage until the end of the fourth fetal month, after which it is nearly always fibrocartilage.
8. Interauricular bands were present throughout fetal life and early infancy and may be found in the adult.
9. Passive motion in the joint increases after the sixth month and is greatest at term, after which it lessens, although the cavity is preserved throughout life, even when extra-auricular synostosis has occurred.

This investigation was undertaken at the suggestion of Dr. A. W. Meyer, to whom the author acknowledges his indebtedness for helpful and stimulating criticism.

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# CRITERIA OF PLACENTAL ENDOCRINE ACTIVITY IN THE MOUSE

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ONE TEXT FIGURE AND TWO PLATES (FOUR FIGURES)

The suppression of oestrus in the mouse during placental retention (Newton, '35) implies a direct or indirect relationship between placenta and ovary. In man, monkeys and some other animals in which the ovaries are unnecessary for continued gestation after the early stages of pregnancy, a case can be made out for a straightforward depression of the ovaries, particularly as these may show atrophic changes. No such simple relationship can be postulated for the rat and mouse, since the ovaries of these animals cannot be removed without terminating pregnancy.

Klein ('35) has shown very clearly that in the rat with retained placentae mucification of the vaginal epithelium is dependent on the presence of the placentae, but cannot be maintained in the absence of the ovaries. From this and previous work on the endometrium of the rabbit ('33), Klein suggests a stimulating action of the placenta on the corpus luteum. However, there have been indications that the placenta itself may also produce progesterone, or exert an endocrine influence independently of the ovary. Thus Selye, Collip and Thomson ('35) on the grounds of endometrial changes and maintenance of the mammary glands in rats, and Haterius ('36), who succeeded in maintaining gestation in oöphorectomized rats from which all except one foetus, but no placentae, had been removed, suggest that the placenta secretes a small amount of progesterone (see also McKeown and Zuckerman, '38, concerning suppression of oestrus).

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The work reported in this communication was undertaken with the object of adding to the three criteria of placental activity in the mouse previously reported, namely, suppression of oestrus, maintenance of body weight and the occurrence of parturition at the proper time (Newton, '35). The state of the mammary glands was an obvious choice, particularly as they are very sensitive in the mouse, uniform in their response, and capable of rapid involution. In addition, the ligamentous transformation of the symphysis pubis, which normally accompanies pregnancy (Gardner, '36), was studied. These criteria having been established in mice with retained placentae, the effect of oöphorectomy was investigated.

## METHODS

The foetuses were destroyed on the twelfth day of pregnancy by the method already described (Newton, '35), i.e., bursting the amniotic sacs by digital pressure under ether anaesthesia; or when the uterus was slit, they were removed surgically. In this investigation, the day on which the seminal plug was found was taken as zero in recording the day of gestation.

The ovaries were removed by the lumbar route, except when the uterus was opened, when they were removed through the median anterior abdominal incision. For the simpler operations such as foetal destruction and oöphorectomy, ether anaesthesia was used, but in the more severe operations sodium amytal was given as well (0.15 cc. of a 1% solution per 10 gm. body weight).

At autopsy on the eighteenth day the length of the interpubic ligament was estimated by putting it on the stretch and measuring the shortest distance between the bony points of attachment. The mammary glands were prepared by skinning the animals, stretching the skins uniformly on boards of the same size, and fixing in Bouin's fluid. The glands were then dissected from the skin, stained in Delafield's hematoxylin, dehydrated and mounted in dammar. Paraffin sections were prepared of the same portion of each fourth gland. As a standard of reference for the whole mounts, the third gland (occasionally the second) was used (fig. 1).

The uteri and placentae were fixed in Dustin's fluid and paraffin sections made for the purpose of confirming the presence of living placental tissue and recording the state of the endometrium. Records of the body weight of the animals were kept from the beginning of pregnancy to the end of the experiment.

## OBSERVATIONS

### *The mammary glands*

To avoid the use of an arbitrary quantitative classification of the state of the mammary glands, such as the use of a varying number of positive and negative signs, a silhouette of the fringe of a lobule of each third gland has been drawn with the aid of a camera lucida, and the drawings reproduced in figure 1. Photomicrographs of a larger portion of four representative glands are shown in figures 2, 3, 4 and 5. The mammary glands of mice are very uniform, both in different parts of the same gland and in different glands, and the various reproductions may be taken as truly representative. The mice are referred to throughout by the letter of the alphabet over which their glands appear in figure 1. Observations fall into three main groups, as follows:

*1. Destruction of foetuses without oöphorectomy.* The foetuses of mice A to E were destroyed on the twelfth day of pregnancy (in one case on the eleventh) and the mothers were killed on the eighteenth or nineteenth day. The presence of the placentae was confirmed by abdominal palpation under ether anaesthesia on each intervening day, though delivery had taken place in the 24 hours preceding autopsy in mice B and C. All the mammary glands were indistinguishable from normal full-term glands. They were very dense and their alveoli were distended with secretion (fig. 2). In section, the

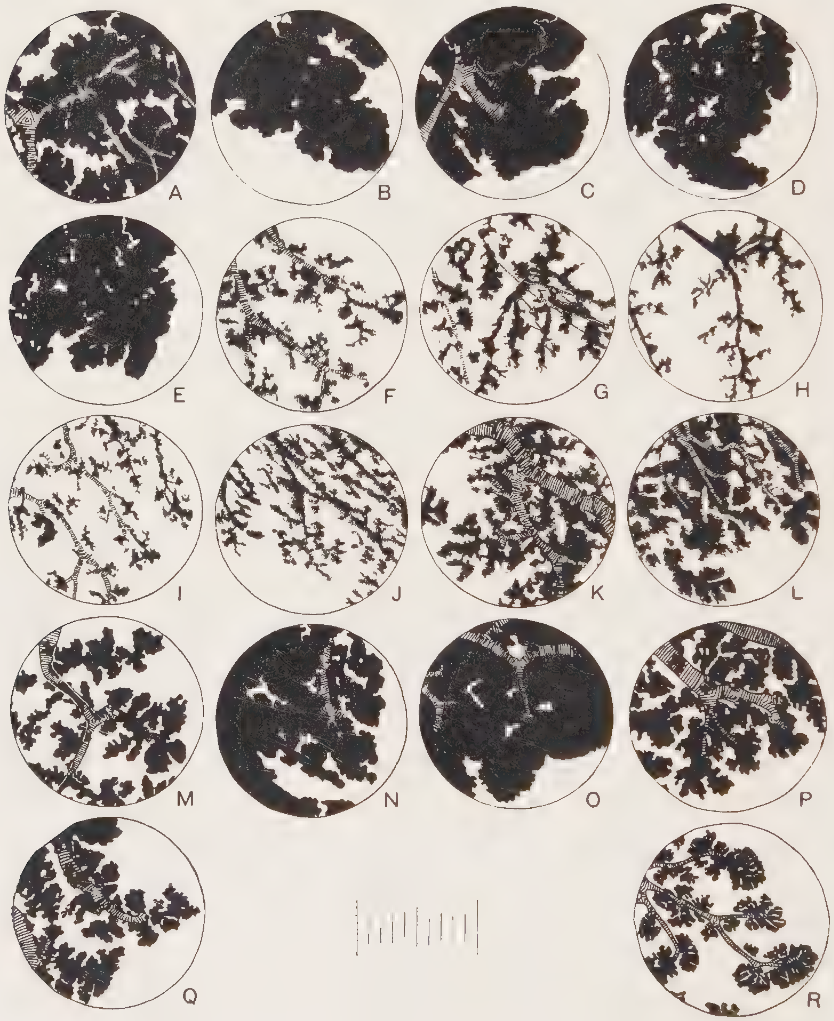

Fig. 1 Silhouette drawings of the fringe of the third mammary gland (the second in the case of mouse J) of each mouse described in text. Ducts are hatched except in H and J. Fine hatching of the outline of the glands F to L indicates structural disintegration. Involution not generalized in mouse L. Uniform magnification. Small divisions of scale = 0.1 mm.

cells of the alveoli were seen to contain large vacuoles, and the glands of mice B and C appeared to be the most active.

2. *Foetal destruction and oöphorectomy, followed by loss of living placental tissue before autopsy.* a. In mice F to J the foetuses were destroyed on the twelfth day and oöphorectomy was performed on the twelfth, thirteenth or fourteenth day. By the sixteenth day, abortion of placentae had occurred in each case and was then complete except in mice F and H. In mouse F three bodies, very small and unlike placentae, were palpated up to the seventeenth day. Had the stools not happened to be fluid they would have been regarded as probably faeces. Unfortunately, at autopsy on the eighteenth day the uterus was empty. In mouse H, two small necrotic placental fragments were found attached to the uterus. One contained a few live giant cells undergoing abnormal and abortive divisions, and showing vacuolation of chromosomes. All the mammary glands in this group showed extensive involution (fig. 3).

b. Mice K and L underwent foetal destruction on the twelfth day and were oöphorectomized respectively on the fourteenth and twelfth days. In both, placentae were present in the uterus on the seventeenth, but not at autopsy on the eighteenth day. In mouse K, all except one felt much smaller than normal. In mouse L, the progressive diminution in the number of placentae in the uterus, down to two on the seventeenth day, was accompanied by the appearance and progressive enlargement of a suprapubic tumour. This proved to be a mass of six placentae, all dead at autopsy, impacted in the vagina. The mammary glands of mouse K were quite well developed, but undergoing definite involution (fig. 4). Those of mouse L were better developed, with distended peripheral alveoli, and although involutionary changes were present, some areas appeared free from degeneration. These two animals mark a step toward the more complete mammary development achieved by those described in the next paragraph.

*3. Foetal destruction and oöphorectomy, with retention of living placental tissue till autopsy.* Mice M to Q were subjected to foetal destruction or removal on the twelfth day and killed on the eighteenth day. Mouse M was oöphorectomized on the fourteenth day, and the rest on the twelfth day. Mouse N spontaneously retained eight placentae till autopsy, six of which looked healthy; three of the latter were examined microscopically and proved to be alive. Only the upper placenta of one horn was preserved by mouse M, presumably because it had herniated through the dorsal oöphroectomy incision and was held in a subcutaneous position by the muscle. It was of normal size, and apart from one or two small foci of degeneration, healthy. Mouse O being found in the act of abortion on the fourteenth day, immediate laparotomy was performed and the uterine horns slit longitudinally. The remaining four placentae were retained. This emergency was anticipated in mice P and Q by performing hysterotomy, oöphorectomy and foetal removal at the same operation on the twelfth day, a severe procedure which left the animals visibly depressed for 24 hours. All the placentae of mouse O and three or four from each of mice P and Q were examined histologically. One from mouse O was healthy, the remainder all presented a mixed picture of normal tissue, tissue containing pyknotic nuclei, and necrosis. Mice N and O had fully developed mammary glands (fig. 5), but those of mouse M were little advanced in point of density beyond the twelfth day condition. All three showed distended alveoli, indistinguishable in the whole mount from those of mice A to E. The glands of mice P and Q were intermediate in density and their alveoli were moderately distended. Judged by vacuolation of the cells, as seen on histological section, all these glands showed somewhat less secretory activity than those of mice A to E. No changes of a degenerative nature were found. The ducts were very dilated.

For comparison, a rather well-developed normal gland of the twelfth day of pregnancy has been included in the series (see R, fig. 1).

### *The interpubic interval*

Of ten normal mice pregnant for the first time, three on the seventh or eighth day showed no pubic separation and no movement; four on the twelfth and one on the thirteenth day showed movement or had a separation of less than 0.5 mm.; two on the twelfth and one on the fourteenth day had a separation of 0.5 to 1.0 mm. Gardner ('36) records ligaments 2.0 to 2.5 mm. in length in four primiparous mice the day after parturition, and one of 1.0 mm. in a mouse at the seventeenth day (private communication).

Five mice subjected to foetal destruction without oöphorectomy during their first pregnancy, including mice A, C, and D, had interpubic ligaments 2.5 to 3.5 mm. in length at autopsy on the eighteenth, nineteenth or twentieth day.

Of mice F to Q, all pregnant for the first time and all having undergone foetal destruction and oöphorectomy, none had a measurable interpubic interval, although slight movement of the pubes was possible. There was no difference between those with and those without living placental tissue at autopsy on the eighteenth day.

### *The timing of parturition, maintenance of body weight, and state of the endometrium*

*Parturition.* In the presence of the ovaries, placentae retained after foetal destruction are delivered at normal full term as previously stated (Newton, '35) and since confirmed on numerous occasions.

In fifteen mice, including mice F to N, elimination of foetuses on the twelfth day of pregnancy was accompanied, or followed within 2 days, by oöphorectomy. Placental abortion occurred before the eighteenth day in all except three, of which one (mouse N) was killed on that day. The other two were killed on the twenty-fifth and twenty-second day respectively, and their uteri each contained a mass of impacted placentae, largely necrotic, but containing living tissue. Of the twelve mice which aborted, abortion was incomplete in four cases. Two (mice H and M) were killed on the eighteenth day, their uteri containing respectively two dead and one living placenta. One delivered its last two placenta naturally on the eighteenth day, but the remaining one still retained a single living placenta when killed on the twenty-fourth day. This placenta was fusiform in shape, firmly attached and contained an islet of cartilage and an epithelial 'pearl.' (Mice O, P and Q are excluded from this group on account of hysterotomy.)

*Body weight.* The weight of mice with ovaries is maintained after destruction of the foetuses until after the placentae are delivered, when it falls abruptly (Newton, '35; Brooksby and Newton, '38). In ten of the experiments now reported, there was an interval between foetal destruction and oöphorectomy of 1 to 2 days. The aggregate weight of the mice fell by only 2.2% in this interval. Adding eight mice in which the two operations were performed simultaneously, the aggregate weight of the eighteen animals fell by 10.7% on the first day after oöphorectoomy, when abortion had not yet occurred. The possibility that this initial fall in weight was due to the operation itself and maintained by subsequent placental abortion receives no support from the weights of mice in which abortion was delayed.

*The endometrium.* For satisfactory investigation of the endometrium, a 'sterile horn' is necessary (compare Selye, Collip and Thomson, '35, and Selye and McKeown, '35). Lacking this, our animals yielded no reaction constant enough to be reported. Varying degrees of mitotic activity were, however, observed after oöphorectomy.

## DISCUSSION

Selye, Collip and Thomson ('35) make passing mention that in rats, whose uteri contained placentae only, the mammary glands were well developed and contained secretion when they were killed about a week after oöphorectomy. This was in contrast to the involution which would have taken place by this time had the uterus been completely emptied in the first place. Our experiments are in agreement with these findings and extend them to the mouse.

The probability is that, as far as growth is concerned, the ovaries are unnecessary. The glands of mice N and O were as dense as normal eighteenth day glands, while the small advance beyond the twelfth day condition of those of mouse M could be explained on the basis of a relative deficiency of placental tissue. The intermediate state of the glands of mice P and Q might be due to a similar deficiency, or to the severity of the operation—simultaneous double oöphorectomy, hysterotomy and removal of foetuses and membranes. In view of the statement by Turner and Gomez ('33) that proliferative activity of the mammary glands is almost complete by the twelfth day in the mouse, we hesitate to attach more than a descriptive significance to the word 'growth,' although superficially our observations are more in keeping with those of Cole ('33) who believes that growth is continuous throughout pregnancy.

The precise reason for mammary development after oöphorectomy is obscure. The obvious possibilities range from the direct action of an internal secretion of the endometrium or placenta to an indirect action of such secretions, or of a nervous stimulus, by way of the anterior pituitary. Three such possibilities are unlikely. The first is secretion of oestrogen by the placenta, for oestrogens are the only substances known to produce pubic separation in the mouse (Gardner, '36), and this does not occur in our experiments in the absence of the ovaries. The second is the stimulus of uterine distension, which was eliminated in mice O, P and Q. The third is an internal secretion of the endometrium, for Astwood ('38) has obtained some mammary development in oöphorectomized rats following the injection of placental extracts.

With regard to lactation, our results are inconclusive. The alveoli of the glands of M, N and O are distended to an extent comparable with those of A to E, but the secretory activity of the cells as judged by their degree of vacuolation appears less. The alveoli of P and Q, while appearing full of secretion in the mounts of the whole glands, are seen in paraffin sections to include many in which there is little or no secretion. The relative lack of density of these glands compared with those of N and O could be attributed in large measure to the smaller size of the alveoli. In mice B and C (ovaries present), whose uteri had emptied themselves during the 24 hours prior to killing, secretory activity was enhanced, but in mice K and L (ovaries absent), in which so far as we know the same thing had taken place, degeneration was beginning or in progress and there was nothing to suggest increased secretory activity. While unwilling to stress these observations, which were incidental to our main object, we cannot exclude the ovaries as having some function in the initiation of normal lactation. Otherwise, we have seen nothing to disagree with Nelson's ('34) finding in guinea pigs that the lactation which would normally follow emptying of the uterus is suppressed when the placentae are left in situ, even though both ovaries be absent.

Selye, Collip and Thomson ('34) state that simple uterine distension by paraffin wax is sufficient in rats to inhibit lactation, but Freud and Wijsenbeek ('38) find that slitting the uterus in pregnant rats does not precipitate lactation and that its suppression is primarily dependent on the presence of embryos attached to the uterus. Our observations on mice O, P and Q support the latter investigators, although the influence of distension as a contributory factor is not excluded.

The haphazard delivery of the placentae after removal of the ovaries is in agreement with the observation of Haterius ('36) on rats that parturition failed to occur after oöphorectomy in experiments in which one complete foetus and several placentae were retained in the uterus, and also with the findings of Kirsch ('38) in experiments with rats similar to ours. The placental abortions and other observations also agree with the theory of Selye, Collip and Thomson ('35) that the uterus contracts on its contents after oöphorectomy during pregnancy, though the placentae of rats are evidently better retained than those of mice. In both these animals it seems that the presence of the ovaries is essential for the correct timing of parturition.

We have now a useful list of criteria of placental activity in the mouse. After foetal destruction there are suppression of oestrus, a maintenance of body weight, continued development of the mammary glands, separation of the symphysis pubis, and parturition at the correct time followed by a loss of weight. It is probably permissible to add mucification of the vaginal epithelium, investigated in rats by Klein ('35). All of these, with the important exception of mammary development, are dependent on the simultaneous presence of the ovaries. In the event of its being possible to transplant placentae to a site other than the uterus, fulfilment of these criteria would constitute final proof that the placenta is an organ of internal secretion. The finding by Astwood ('38) that two types of rat placental extract act respectively on the ovaries and mammary glands may render such experiments unnecessary.

Another practical point raised is that in the artificial production of full mammary development in the mouse, it will be necessary to find some means which does not involve the simultaneous formation of an interpubic ligament.

It should be emphasized that our findings are not necessarily applicable to other animals, although the rat seems to be similar in many respects. It is, for instance, a commonplace that in many species pregnancy can be continued and parturition, and lactation take place normally after removal of the ovaries early in gestation. The explanation of such species differences is yet to be found, but at present it seems likely that the 'control' of gestation is vested largely in the placenta. In some animals, such as the mouse, this organ may merely modify ovarian activity, in others such as man, it may supplant the ovaries altogether. It is even possible, as suggested elsewhere by one of us (Newton, '38), that one of the functions of the 'gonadotropic' hormone excreted in the urine of human pregnancy is, after a temporary stimulation of the corpus luteum, to supress the follicular system of the ovary and produce a functional oöphorectomy.

## SUMMARY AND CONCLUSIONS

It has been demonstrated that the presence of placentae in the uterus of the mouse has a positive influence on mammary development which is independent of the ovaries and which is not initiated by uterine distension. The participation of the ovaries and of distension in normal pregnancy is not necessarily excluded.

Retention of placentae also determines the formation of an interpubic ligament at least as long as, if not longer than that found at the end of normal pregnancy. In common with several other phenomena accompanying placental retention which are listed in the text, this is dependent on the presence of the ovaries.

The significance of these findings is discussed.

We wish to thank Prof. E. Allen for his continued encouragement and interest, Dr. W. U. Gardner for much help and advice, and Dr. A. Kirschbaum for valuable technical assistance.

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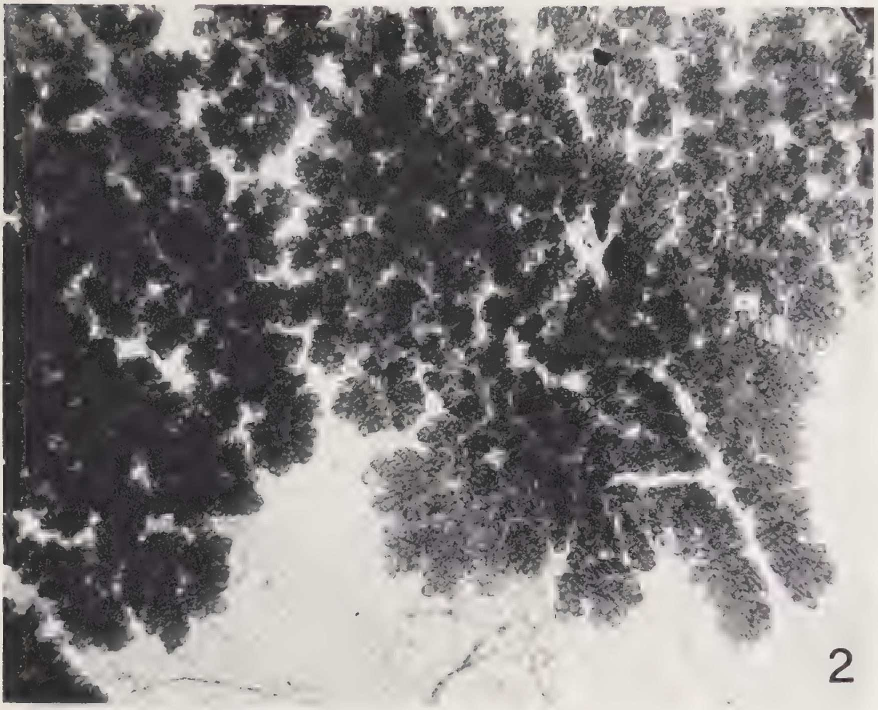
## PLATE 1

### EXPLANATION OF FIGURES

2 Part of mammary gland of mouse A, showing full growth and distension of alveoli. Photomicrograph from whole mount. × 25.

3 Part of mammary gland of mouse H, showing extensive involution. The circle shows the field included in the silhouette of the same gland in figure 1. Photomicrograph from whole mount. × 25.



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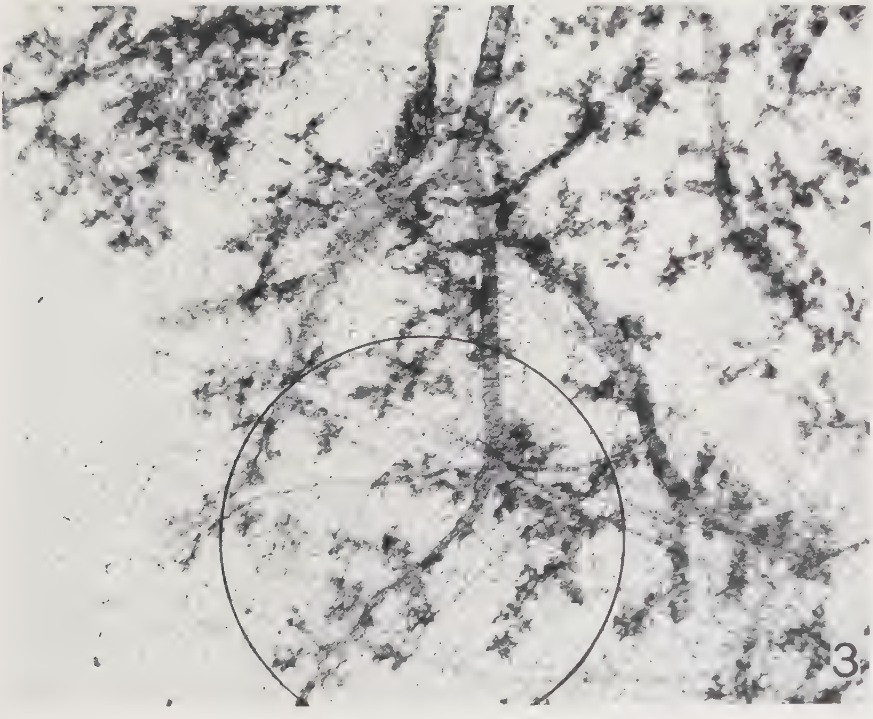

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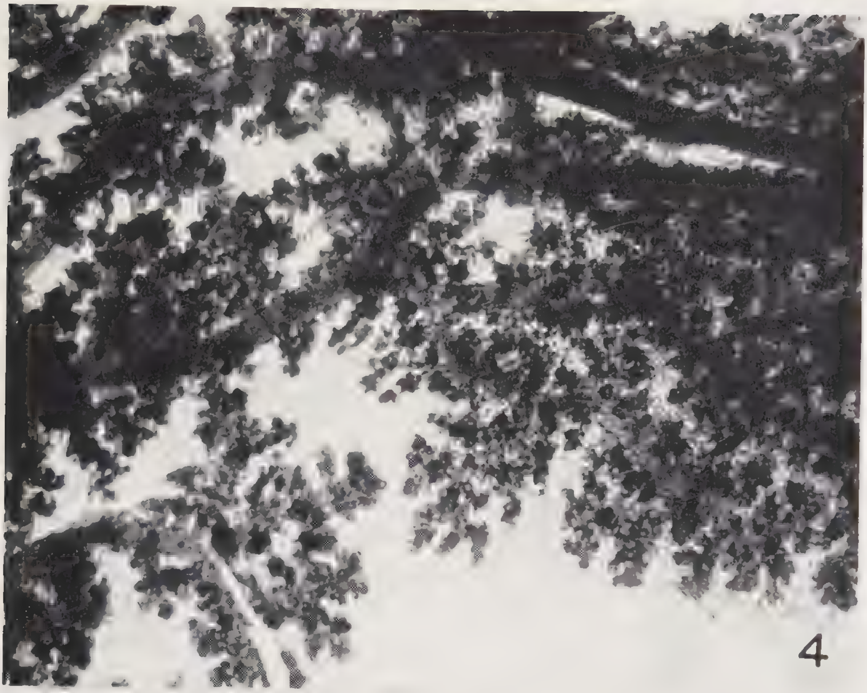
# PLATE 2

## EXPLANATION OF FIGURES

4 Part of mammary gland of mouse K, showing well-developed gland undergoing involution. Photomicrograph from whole mount. × 25.

5 Part of mammary gland of mouse O, showing full growth and distension of alveoli. Photomicrograph from whole mount. × 25.



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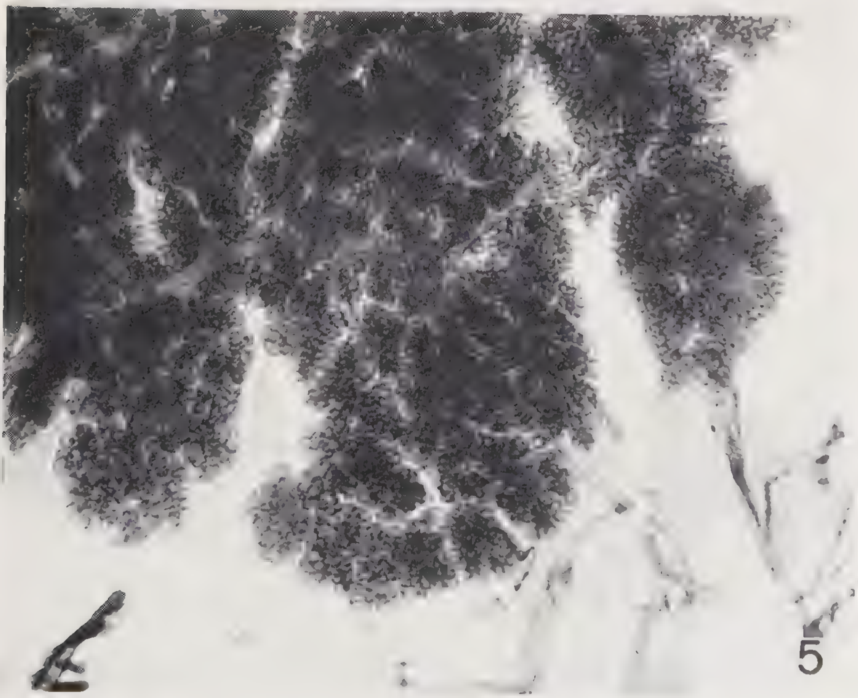

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# PLASMOSIN. THE GEL- AND FIBER-FORMING CONSTITUENT OF THE PROTOPLASM OF THE HEPATIC CELL[1]

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THREE FIGURES

Since the advent of the colloidal idea the biochemistry of protoplasm has receded more and more in importance. This situation arises from the fact that it is extremely difficult to know the actual source in the cell of substances extracted from it, and also from a resonable doubt as to whether substances derived by extraction actually exist as such in the living cell. In the preface to his great work on the chemistry of the plasmodia of Aethalium septicum, Reinke and Rodenwald (1881) remarked, "I have come to the conviction through experiment that a plasmodium rubbed up in mortar is no more protoplasm than a watch reduced to a fine powder is a watch." This thought runs through all of the studies of protoplasm and creates an atmosphere of doubt as to the validity of any of the results obtained by biochemical methods. The discussion of the structure of protoplasm from the purely colloidal standpoint, however, has not been as profitable as was hoped. After 30 years of speculation we are still unable to explain this mysterious organization which resists the centrifuge, the reversible gelation of protoplasm, the formation of spindles and asters, or the nature of the surface membrane.

Protoplasm although consisting of colloids possesses says Gray ('31), "a chemical heterogeneity incomparably greater

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[1] This investigation was aided by a grant from the Rockefeller Foundation.

than that of any of the simple systems studied by colloidal chemists.'' Beams and King ('36) have shown that the application of forces of ultracentrifugation which are adequate to sediment colloidal solutions do not abolish the polarity of the egg of Fucus, and that under really colossal centrifugal force the eggs of Ascaris still continue to show mitosis and to segment. Protoplasm thus posesses an organization which at the present time is not easily explained in colloidal terms, and in the opinion of the writer an interpretation of protoplasm on colloidal principles alone, without consideration of the specific colloids which compose it, has no more chance of success than an interpretation of coagulation of blood would have if fibrinogen, kephalin, etc., were unknown. That this opinion is shared by others is indicated by the recent reexamination of the chemistry of the slime moulds by Lepeschkin ('26) and Kiesel ('30), and of the stroma of erythrocytes by Jorpes ('32) and Boehm ('35).

The time seems to be appropriate for calling attention to the fact, well known by the early biochemists, that protoplasm contains substances which in their own right by reason of their property of producing gels, fibers, and membranes are worthy of special consideration in any discussion of the properties of protoplasm. To this group belong the vitellins of Hoppe-Seyler and of Kossel, the nucleo-albumins of Halliburton and Lilienfeld, the hyalin substance of Rovida, studied also by Miescher, and the plastins of Reinke and Rodewald, Zacharias, Schwarz, Carnoy, and others.

In a recent paper Bensley and Hoerr ('34) called attention to the fact that the liver cell of mammals is extraordinarily resistant to extraction, that it retains its individuality as a cell, and the main features of its structure, even when large quantities of its protein constituents are extracted by the use of dilute salt solutions and weak alkaline solutions. The residue, soluble in alkalis, they termed ellipsin and compared it with the plastins of the authors mentioned above. At the present time the chemical status of plastin is more confused than ever, because Lepeschkin ('26) in his recent studies was convinced that it was largely composed of nucleo-proteins, and Kiesel ('30) was equally sure that it was merely a skeletal substance, peculiar to the fruiting bodies of the slime moulds. But the term plastin, as used by Zacharias ('10), Schwarz (1887) and Carnoy (1884), was applied to a residue left in the cell after artificial digestion with pepsin-hydrochloric acid, and, for reasons which will appear later, was probably complex in composition.

The chemical relationships of these various substances are still obscure, and for that reason Bensley and Hoerr rejected the name plastin and applied the name ellipsin to the substance left after successive extraction with salt solution and weakly alkaline solutions. The chemical nature of this residue is still undetermined and at this time it is only necessary to insist that the protoplasm of the liver cell contains difficultly soluble materials which are grouped under this general name.

During the course of this investigation Bensley and Hoerr found that a suspension of liver cells repeatedly washed with 0.85% sodium chloride solution until the washings contained no detectable protein showed little structural change visible under the microscope, although the cells had lost about 70% of their total solids. When these washed cells were treated with an N/200 solution of ammonia in 0.85% sodium chloride, they immediately adhered to one another and began to swell until after the lapse of 24 hours they had been transformed into an opalescent gel. It was thought that this was due to the swelling of the cells themselves but later investigations have shown that if the emulsion were first thoroughly extracted with a 10% solution of sodium chloride the gel transformation no longer took place on the application of alkaline salt solution. The cells remained separate and substances which were soluble in these alkaline solutions were then extracted. The formation of gels in the cell suspension, therefore, was due to the presence of a substance which could be extracted from the washed cells by 10% sodium chloride solution. This substance is the substance to which I have given the name, plasmosin.

The behavior of this solution is similar to that previously described by Rovida, Miescher, Halliburton, Lilienfeld, and others, for the substances which have been variously referred to as vitellins, nucleo-albumins, phospho-proteins, etc. Whether these substances constitute a chemically-homogeneous group is not definitely known, but from the standpoint of the physical properties of protoplasm they may be so considered. Since these terms leave one in doubt as to the intracellular origin of the substances in question, it seems to me to be preferable to call attention to the cytoplasmic origin by the use of a new name, and for that reason I have called the substance obtained from liver cells by extraction of washed cells with 10% sodium chloride, plasmosin, in the hope that by means of this name its cytoplasmic origin may be emphasized.

Before proceeding to a description of the properties of plasmosin from liver cells it may be well to briefly review the literature.

Rovida (1867) published an account of the changes in colorless blood corpuscles, bone marrow cells and frog blastomeres when treated with 5 to 10% solutions of sodium chloride. In all of these cases he was struck by the appearance of hyaline droplets on the surface of the granular protoplasmic body. He said that the cells shrank after the addition of such solutions but after a variable lapse of time swelled again. During this process of shrinkage and swelling hyaline droplets appeared on the surface of the cell which remained for a long time, even up to 2 days, sticking to the cells, and then apparently slowly dissolved.

The following year Miescher (1868), in connection with his classical studies of the composition of nuclei, confirmed Rovida's observations and studied the properties of the hyaline substance. He called attention to the well-known property of pus of forming turbid, slimy gels when treated with common salt solutions of 3 to 10% or over. These gels were precipitated by water and were compared by some to myosin, and by others to fibrin. According to Miescher, after

24 hours' action of a solution of sodium sulphate many pus cells showed a hyaline margin, in some cases on one side, forming hemispherical processes, and, in others, surrounding the whole cell. In sodium chloride solutions the hyaline portions increased more and more in volume and their contours at first clear became paler and finally disappeared altogether. The slimy mass derived as an end product of this action was never distributed uniformly in the excess sodium chloride. It was not a true solution. The gel was precipitated in shreds by the addition of water and he recognized between the granular unchanged cells a fibrous membranous mass which cemented the cell remnants together. This picture was not altered by days of action of much distilled water. The precipitated shreds treated with salt solution gave again the gel unless they were kept too long under distilled water, in which case swelling in sodium chloride no longer occurred. The substance was soluble in very dilute hydrochloric acid or soda solution.

Plosz (1873) applied similar methods to the study of the proteins of the liver. He extracted the liver cells first with 0.75% salt solution and obtained an extract containing a) a protein which began to coagulate at 45°C., b) a nucleoalbumin which coagulated at 70°C. Further extraction of the cells with 10% sodium chloride solution gave quantities of albumin coagulating at 75°C. This was reprecipitable by dilution and also by excess of concentrated sodium chloride solution. This protein was also precipitable by addition of hydrochloric acid and was digested without residue by pepsin. The residue of the cells after extraction with the salt solutions was insoluble in water, in neutral salt solutions, and immediately soluble in sodium hydroxide.

Plosz does not appear to have noticed the tendency of the liver cells to form gel-like masses in 10% sodium chloride solutions.

In 1887 and 1888, Halliburton reported for a committee consisting of Schäfer, Foster, Lankester and himself, the result of his investigations on the composition of lymph cells. He said that after thorough shaking of the cells in a solution made by mixing a saturated solution of sodium sulphate with nine times its volume of distilled water, the cells dissolved to a very great extent and microscopical examination of the debris showed that it consisted chiefly of nuclei, with pieces of shrunken protoplasm adhering to or separating from the nuclei. He distinguished two globulins, one coagulating at 48 to 50°C., and another coagulating at 75°C.; two albumins, coagulating at 73 and 80°C., respectively; albumose and peptone; and, finally, a proteid similar to that described by Miescher in pus, which swelled up into a jelly-like substance in solutions of sodium chloride and magnesium sulphate. When the cells (lymphocytes) were extracted with a 5 or 10% solution of either sodium chloride or magnesium sulphate, the result was a slimy mass, very much resembling mucus in its appearance. By pouring this mass into water in large excess the protein precipitated in cohesive strings and in time these contracted and settled at the bottom of the water. This preciptate was washed with 0.75% sodium chloride solution in which it was very slightly soluble. It had the following properties: a) insoluble in water, b) slightly soluble in 0.75% sodium chloride solution, and c) soluble in 10% sodium chloride solution. When the latter solution was heated to 50°C. the substance shrank and could be filtered off, but if the solution was boiled the substance dissolved again. It was precipitated by dilution with water or by the addition of acetic acid; in the latter case in strings like mucin. He said that the substance was undoubtedly a constituent of the cell protoplasm.

In his second report on lymph cells Halliburton confirmed his previous report and added that the properties of the protein which we have just been discussing led him to conclude that it belonged to a class of proteins called by Hammarsten nucleo-albumins. He prepared the substance as before and found that when washed and kneaded in distilled water, and finally washed with rectified spirit and absolute alcohol it yielded on ignition an ash rich in phosphorus.

In 1892 Halliburton made a study of the proteins of the liver and the kidney. In the former cases he confirmed Plosz' work in general but got rather different results as to the heat coagulation points of the globulins extracted with saline solution. He found 1) a protein which coagulated between 45°C. and 50°C., 2) a protein which coagulated between 56°C. and 60°C., 3) a protein which coagulated between 68°C. and 70°C., and 4) a protein which coagulated between 70°C. and 72°C. He used for extraction a 5% solution of manganese sulphate. Of these the one which coagulated at 56°C. to 60°C. left, when digested in pepsin and hydrochloric acid, a residue which contained a considerable quantity of phosphorus.

From the kidney extracted with 5% magnesium sulphate he obtained two proteins, one a globulin which coagulated at 52°C., and the other a nucleo-albumin which coagulated at 63°C.; the latter he said was the most abundant protein and was derived, not from the nuclei, but from the protoplasm of the cells.

He found also that if he ground up kidney in a mortar with saturated sodium chloride solution the result was a slimy, viscous mass. On pouring this mass into an excess of distilled water the nucleo-alubumin separated out in strings and floated to the surface where it could be collected.

The method for separation of the kidney nucleo-albumin was unsuccessful in Halliburton's hands when applied to liver. Halliburton identified these nucleo-albumins with the tissue fibrinogen of Wooldridge and claimed that they could be prepared also by the method of Wooldridge by extraction of tissues with water and precipitation with excess of acetic acid. Like Wooldridge's preparations, when dissolved in sodium carbonate and injected intravenously, they caused intravascular clotting.

Lilienfeld (1894), in his studies on the chemistry of lymphocytes, also found a substance which he obtained by extraction with 10% salt solution. This substance was incompletely digestible in pepsin-hydrochloric acid, giving a residue insoluble in dilute acids but easily soluble in caustic and alkaline carbonates. This protein after extraction with warm alcohol, cold absolute alcohol and ether contained 0.433% of phosphorus. The nucleo-histon which he obtained from lymphocytes by extraction with water and precipitation with acetic acid contained about 3% of phosphorus.

It is interesting to note that Mills ('21) who prepared tissue fibrinogen from dried lungs by extraction with 0.9% sodium chloride solution and precipitation with acetic acid found that the substance extracted consisted of 41.6% of phospholipin and 58.4% of protein; the latter contained about 1.06% of phosphorus.

Recently Boehm ('35) has prepared a ropy gel from calves' blood corpuscles, obtained from oxalated plasma, washed repeatedly by centrifugation and then hemolyzed by distilled water. The agglutinated stromata were then collected and treated with a mixture of lithium chlorate (1.25 m.) and double concentrated borated buffer in equal parts to give a pH of 7.7. In this solution the stromata dissolved to a ropy gel.

These investigations, showing as they do the presence in protoplasm from several sources of a special substance capable of forming ropy gels with stronger sodium chloride solutions, raise the question as to the general presence in protoplasm of such substances. In this connection we may refer to the original work of Reinke and Rodewald (1881) on the plasmodium of Aethalium septicum. The more recent investigations of Kiesel ('30) and Lepeschkin ('26) on plasmodia of various slime moulds unfortunately give us no information on the fundamental proteins of the protoplasm, since their material was collected in water containing alcohol or ether or in pure alcohol, and so was unsuitable for extraction experiments. Reinke and Rodewald, on the other hand, studied fresh protoplasm. By pressing in a narrow-meshed linen filter press they obtained from 179 gm. of protoplasm 120 gm. of a turbid yellowish fluid, leaving behind a firm, moderately dry cake of solids. This juice coagulated at a temperature of 48 to 64°C., yielding a coagulum of about 7 to 8% of the total weight of the protoplasm.

Quite fresh protoplasm was rubbed up in a 10% solution of sodium chloride and filtered. This solution was yellow in color, had an alkaline reaction, and coagulated on heating. Saturation with sodium chloride produced, but in a relatively small quantity, a flocculant precipitate of a substance which the authors called myosin. Carbonated water added to the sodium chloride solution in great excess produced a copious precipitate, which the authors called vitellins, the filtrate from which contained no further coagulable protein.

It is thus apparent that even in the primitive protoplasm of the slime moulds there is present a protein constituent which is soluble in high concentrations of neutral salts and precipitated from it by dilution with slightly acidified water.

To prepare plasmosin from guinea pig liver the liver is first freed of blood by perfusion from the aorta with cooled salt solutions. The salt solution should be neutral, since slightly alkaline solution of a pH 7.4 extracts more material from the cells during perfusion. For this purpose I have employed a simple solution of sodium chloride (0.85%). When the liver is freed of blood it is removed from the animal, the gall bladder is stripped off and the excess of connective tissue at the transverse fissure removed. The tissue is then broken up in a mortar, diluted with salt solution, and pressed first through cheese cloth to break up the masses, and second through bolting silk to divide the smaller masses into their individual cells. Much of the broken-up reticulum goes through the silk. By this means a suspension is obtained containing intact cells, fragments of cytoplasm, mitochondria, fat globules and nuclei. The suspension so obtained is separated in a centrifuge and washed repeatedly in salt solution, cooled to a temperature of about 2°C. This is continued until the wash water contains no protein detectable to the simple precipitation tests.

During this extraction the cells lose about 70% of their dry weight, but the changes visible under the microscope are insignificant. The membrane of the cell remains intact, the mitochondria are unaffected, and the nuclear chromatin scarcely shows visible change. That some extraction of the nucleus takes place is, however, apparent from the fact that the proteins contained in the extract when flocculated by heat show a Feulgen reaction to which the cell protoplasm in the intact state is negative. This extraction of the intact cells does not completely remove the soluble globulins of the cell but very little is left. This may be determined by collecting the residue by centrifugation, grinding it up with sand, and re-extracting it, when the additional yield of globulins soluble in sodium chloride solution will be obtained but in very small amount.

After this washing is complete the residue is extracted with 10% solution of sodium chloride, cooled to 2°C. As soon as this stronger solution of sodium chloride is added the cells become sticky and tend to adhere into a mass, but by frequent shaking the cells may be separated and more complete extraction may be obtained. This extraction is continued for 24 hours in the refrigerator at low temperature with frequent shaking. The reason for maintaining it at low temperature, in addition to the protection from bacterial invasion, is that the substance extracted is highly labile and the yield is much improved by the use of low temperatures.

At the end of 24 hours the material is cleared of cells in the centrifuge and the supernatant fluid vigorously centrifuged until it is only slightly opalescent and can be cleared no further by centrifugation.

It should be noted that after this second extraction the cells of the liver still show little evidence of change, many of the cells are unchanged in size, others are smaller by loss of substance, but the mitochondria are well preserved and so are the nuclei. In the early stages of this extraction the cell first shrinks and becomes irregular, but presently swells again and may become for a time much larger than their original size, but as substance is lost they diminish in size again. Staining the preparation at the swollen stage with methyl green, the nuclei are found to be brilliantly stained as in the fresh cells, but the protoplasm has become metachromatic to this dye and stains a dull red color. This property is due to the swelling of intraprotoplasmic plasmosin. Even the free nuclei retain their staining properties and show little loss of chromatin, indicating that the membrane of the nucleus of the liver cell is a strong barrier to extraction with salt solutions. In some of the cells vacuoles may be seen, but in general although in this extraction an additional 6% of the dry weight has been removed the change microscopically considered is slight.

The clear solution obtained by centrifugation from this extraction contains plasmosin. If the residue of cells be now treated with N/200 ammonia in 0.85% sodium chloride solution, the cells do not stick together nor are any gels or slimes formed. Clearly, the extraction with 10% sodium chloride has removed the slime and gel-producing substance.

It should be noted at this point, that the cells even after extraction with this alkaline solution are still intact. They are, however, much reduced in size, their mitochondria has been removed, their chromatin has been extracted, the protoplasm is full of minute vacuoles so as to give it a honeycomb appearance, but the nuclear and cellular membranes are intact. Inside the nuclei are shreds of undissolved non-chromatin material. The cells have now lost their elasticity and are highly fragile, and may be broken up into fragments by vigorous shaking or too vigorous centrifugation. This residue dissolved in cooled N/1 sodium hydroxide solution and precipitated by acidification does not precipitate in in fibrous form but flocculent form. This is the true ellipsin. The original preparation of ellipsin prepared by Bensley and Hoerr contained also plasmosin, the gel and fiber-producing substance.

From the solution in 10% sodium chloride the plasmosin is precipitated by acidification, or by dilution with water, or by dialysis against distilled water. Precipitation by dilution or by dialysis is preferable because it is less open to the suspicion of producing chemical change. On account of the fibrous form in which the material precipitates the water should be added from the bottom and then slowly mixed with the salt solution by agitation; otherwise the fibrous masses which are precipitated capture air and are floated to the top and are difficult to separate by centrifuging. The precipitation by dilution is contingent upon the reaction of the combined solutions. If the plasmosin is buffered by the addition of phosphates to a pH of 7.4, precipitation on dilution does not take place, which probably explains why Reinke had to use carbonated water to precipitate his alkaline extract from plasmodium.

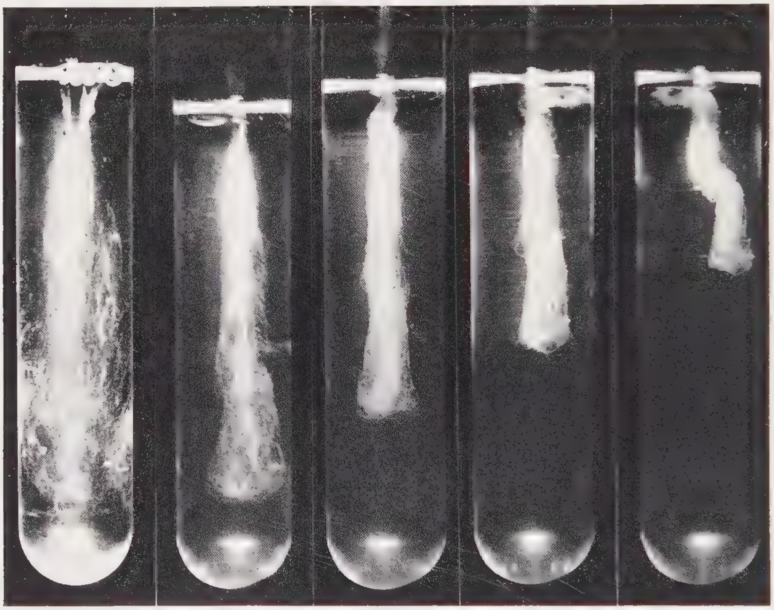

Fig. 1 Stages in the formation and contraction of the plasmosin clot. Lapse of time, 45 minutes.

To precipitate plasmosin from the solution of sodium chloride the addition of about four volumes of distilled water is necessary. When the distilled water is added to the solution of plasmosin the latter first becomes opalescent, then minute fibers make their appearance which quickly join to a fibrous network which slowly contracts. The stages of this contraction are illustrated in figure 1, a series of photographs showing the formation and contraction of the precipitate in a test tube. This contraction reminds one strongly of the contraction of fibrin in a blood clot. The precipitate examined under the microscope consists of fibers of incredible length and smooth contours. They vary in size but the larger fibers may be split by dissection with a pair of needles into smaller fibers and under the dark-field condenser the composite character of the larger fiber may be clearly discerned.

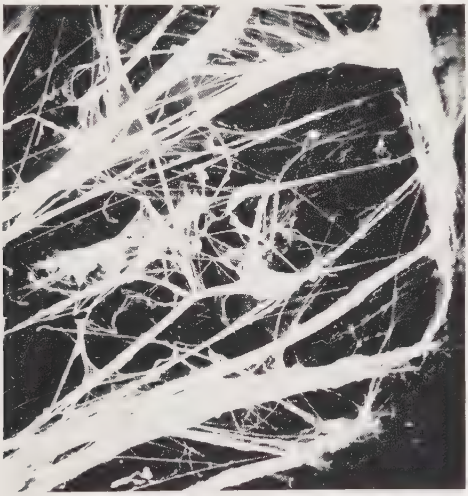

Fig. 2 Dark-field photomicrograph of plasmosin fibers. × 26.

Some fibers are so tenuous that their diameter can be little more than that of the micelli which compose them. The appearance of this fibrous mass as seen under the microscope is shown in figures 2 and 3. The material obtained by precipitation in the manner described may be further purified by re-solution in 10% sodium chloride and reprecipitation in the same fashion. But certain difficulties make their appearance as soon as this is attempted.

Miescher pointed out that the plasmosin extracted from pus corpuscles lost its solubility if allowed to stand under distilled water for a prolonged period. The plasmosin obtained from liver cells is equally labile and the attempt to re-dissolve it in sodium chloride may result only in the formation of a gel which refuses to go into complete solution. If the operations of preparing the plasmosin are carried out as rapidly as possible and if all solutions are kept cool, it may be possible to re-dissolve and re-precipitate the first product, and by this means get rid of some of the residue of the globulins of the liver cell which adhere to the precipitate.

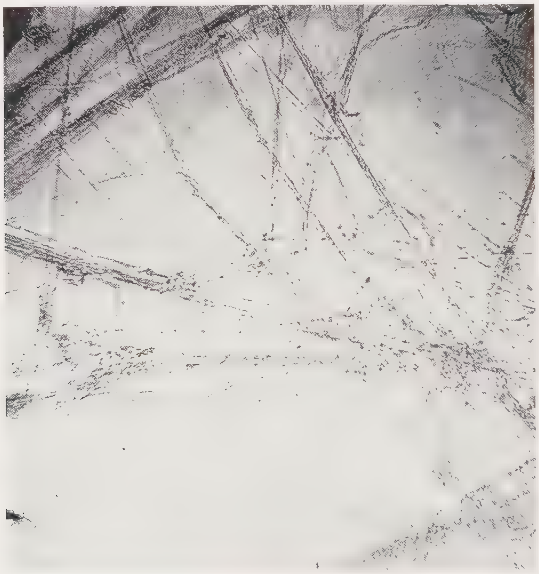

Fig. 3 Photomicrograph by transmitted light of freshly-formed plasmosin fibers, showing their composite character. × 26.

Another method of further purification is by using alkalinized sodium chloride solutions. The addition of alkali will cause a preparation which has already become insoluble in neutral sodium chloride solution to re-dissolve, probably, however, not without significant chemical change.

Plasmosin prepared in the way described is, of course, not a pure product because it is impossible to completely free it from other proteins which adsorb to it, and because also extraction of the nucleus takes place to a minute degree which confers upon the plasmosin the capacity to give a positive Feulgen reaction. To separate this small amount of nucleoprotein from the plasmosin does not seem to be possible.

After precipitation with water or with acid as the case may be, the plasmosin is collected, repeatedly washed in distilled water, and dried in vacuo over phosphorus pentoxide. This preparation, although it has been precipitated from a water-clear or very slightly opalescent solution, contains a considerable amount of fat. I have already reported on the fat content of one preparation of plasmosin which contained 25.8% of total fats of which 1.16% was lecithin and allied substances, 0.46% cholesterol, and 24.18% triglycerides. These quantities are, however, not constant as the fat present may vary from 8% to 31% of the weight of the preparation. The phospholipin is consistently low in all the preparations due to the use of 10% sodium chloride for extraction, which leaves the lecithin of the cell adsorbed to the cell residue. The fat is probably not in combination with the plasmosin but merely adsorbed. This is indicated by the fact that the fat content of the preparation is considerably reduced by filtering it through a Berkefeld filter.

The plasmosin freed from its fat by extraction in a Soxhlet apparatus gives the usual protein reactions. This fact, however, is not of much significance inasmuch as every preparation is somewhat contaminated with both cell globulins and nucleo-protein. The preparation is not completely digestible in pepsin hydrochloric acid, agreeing in this respect with the preparations made by Halliburton and Lilienfeld.

The chemical characterization of this product has not yet been possible but it may be recalled that both Halliburton and Lilienfeld considered that the preparations obtained by them from lymph cells belonged to the class of phosphoproteins. These preparations, however, unquestionably were contaminated with other cell proteins, with nucleo-proteins, and adsorbed inorganic phosphates. It is difficult, therefore, to draw any conclusions as to their chemical nature.

The question of prime importance is the location of plasmosin in the intact cell. As will be recalled, Miescher, Lilienfeld and Halliburton and Kossel were all of the opinion that it was derived from the cytoplasm and not from the nucleus, although Halliburton used the term nucleo-albumin to describe his product. This conclusion I can now fully confirm. The study of the different stages of extraction of the cells shows beyond doubt that the plasmosin belongs to the apparently structureless ground substance of the cell, not to membranes, mitochondria or nuclear chromatin. This conclusion is confirmed by the study of preparations made by the freezing-drying method or the molecular desiccation method as it was called by Harris ('12, '13), and Shackell ('09), whose pioneer work in this field owing to the fact that it dealt with immunological problems we have hitherto overlooked.

Guinea pig liver frozen in liquid nitrogen and dried at a temperature of —40°C. may be used for the preparation of plasmosin. All that is necessary to demonstrate the presence of plasmosin in this material is to cut a free-hand section, place it on a drop of 10% sodium chloride on a microscopic slide, and after a lapse of 5 minutes dilute this drop of sodium chloride with distilled water. The cells float apart and in the interval between them the typical fibers of plasmosin are seen. This is as near as we can get to the demonstration that plasmosin exists as such in living cytoplasm and is not a product of spontaneous change of other substances.

That the plasmosin is not derived from mitochondria may be demonstrated by separating the latter by the method described in previous papers and treating the mitochondria themselves, free from other cell products, with a 10% sodium chloride solution. They remain unchanged in this solution and do not yield a substantial amount of plasmosin to the sodium chloride. Whether plasmosin is also contained in the nucleus, we have not been able to determine, since we have not been able to separate the nuclei from other cell products in sufficient quantity to extract for this substance. The plasmosin is thus unquestionably a component of the optically structureless ground substance of the protoplasm, and it may be inferred that some of the properties of this ground substance are due to its presence.

The fact that plasmosin when it is precipitated from its solutions in strong sodium chloride by dilution readily undergoes a change in solubility which makes it no longer soluble in 10% sodium chloride solution raises the question as to whether the membranous and other insoluble portions of the cell which constitute what is called ellipsin are products of such an irreversible change of plasmosin. This question cannot be answered at present because it requires a complete chemical characterization of both substances for which the opportunity and equipment have not yet been available but which I hope to arrange in the near future.

The relation of plasmosin to the physical properties of protoplasm needs no discussion. It is obvious that in the properties of plasmosin we have at once a plausible explanation of such phenomena as reversible gelation, thread formation, etc., as well as the formation of irreversible thread forms in cells such as tonofibrils, etc. Whether this is the true explanation of these phenomena I am unable to say, but it is at least more plausible than the far-fetched adaptations of the general properties of colloidal solutions.

The nature of the micelles of plasmosin is as yet unknown but since the presence of it confers a certain degree of viscosity on the solution, since they readily collect into long fibers, it seems probable that they are of linear shape. The formation of the fiber results from the end-to-end orientation of these linear micelles. In protoplasm in the liquid state these micelles are probably independent and irregularly arranged but in flowing protoplasm they would be oriented parallel to the axis of flow as described by Seifriz ('31) for the micelles of his phaneroplasm. From this state by simple end-to-end combination all changes in viscosity are possible up to the formation of a fibrous gel as maintained by Seifriz ('29) or even discrete fibers such as are seen in vitro (fig. 3).

At present only two conditions are known which bring about these changes in vitro, namely, change of pH toward the acid side or changes of salt content or kind in the solvating medium. In the complex equilibrium of protoplasm other factors may be operative such as electrical potential differences, etc., but these await further investigation.

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# REGENERATION OF TESTIS IN THE FOWL, AND ITS BEARING ON GERM-CELL THEORY

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ONE TEXT FIGURE AND ONE PLATE (SIX FIGURES)

Among the arguments advanced to support his germ-plasm theory Weismann has written ('04, vol. 1, p. 411) that "Even the familiar fact that the excision of the reproductive organs in all animals produces sterility proves that no other cells of the body are able to give rise to germ cells; germ-plasm cannot be produced *de novo.*" Although sterility following gonadectomy hardly proves that somatic cells cannot or indeed have not transformed into germ cells, the development in the adult animal of a gonad de novo containing definitive germ cells would provide unequivocal evidence of the capacity of somatic cells to form germ cells.

In the present paper are recounted observations which are believed to establish regeneration of testis in the fowl following complete bilateral castration, and in certain cases evidence is presented for the formation of mature spermatozoa from cells previously differentiated as somatic cells. A preliminary report of these findings was made before the American Association of Anatomists in April, 1936 (Hooker, '36).

## OBSERVATIONS

Over a period of 5 years we have had occasion for various reasons to castrate approximately 500 White Leghorn cockerels. In performing these operations it occasionally happened that a testis was inadvertently divided with the result that a fragment was left in the animal. In such cases growth of the comb and other head furnishings invariably was resumed within a month after operation, indicating that the fragment had hypertrophied, attaining a functional level comparable to that prior to operation. In a few cases, on the contrary, comb growth was not resumed until 6 months to 2 years after castrations which had been recorded as complete. The great delay in resumption of comb growth in these cases, as contrasted with the much earlier growth in birds known to have been incompletely gonadectomized, aroused our suspicion that we had encountered de novo development of testis subsequent to total bilateral castration.

Upon the accumulation of five cases of the latter class a study was made to determine the correctness of this suspicion. The study consisted in an attempt to determine whether the original castration had been complete, and to ascertain whether the testes in the two types of animals present any distinguishable differences. While the circumstances preclude any one test serving as an infallible index, several points have been assembled which strongly suggest that these five animals have shown de novo testis formation.

At each castration the operator recorded his impression of the completeness of the removal of gonad tissue, and during the last 2 years all testes taken at operation have been fixed for histological study. From three of the five animals in question the testes removed at the original operation have been preserved. Upon examination with a dissecting microscope these testes present no breaks which would be present if any fragment, however small, had been left in the animal. It seems that no further information could be gained by sectioning these testes.

Another evidence of the totality of the original castration may be had from a study of the relation of the testis to the peritoneum. The various conditions which might obtain are shown schematically in figure 1, in which each diagram represents a transverse section of a lateral half of the animal at the gonad level. In a the relations in a normal bird are indicated; the testis is suspended by a mesorchium from the renal peritoneum. In total excision of the testis a cut is made through the mesorchium at level To, whereas if a fragment is left, the cut will be made at a level similar to that at S. In a subtotal excision the fragment will be at the end of the mesorchium; and when hypertrophy occurs the testis will be pendant in the coelome, suspended by a mesorchium as shown in b. If the removal has been complete, the condition upon healing will be that shown in c. Should it happen that the peritoneum in the adult retains gonad-forming capacity, any gonad which might be produced in the adult would presumably arise in a manner similar to that followed in the embryo; namely, by proliferation of sex cords from the peritoneum, with the cords formed in a retroperitoneal position. A gonad arising in this fashion would occupy the position shown in d. If this gonad were to undergo any considerable growth, the relations would be of the type shown in e, since the peritoneum is more or less taut while the kidney is capable of deformation.

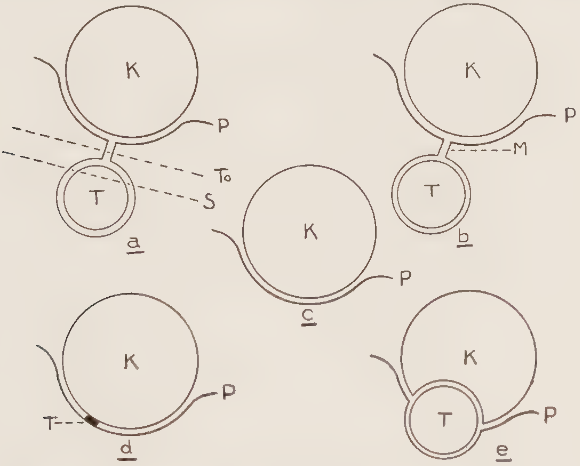

**Fig. 1** Diagram of a transverse section of a lateral half of the animal at the gonad level. Explanation in text. K, kidney; P, peritoneum; T, testis; M, mesorchium.

A large number of animals which have shown comb growth subsequent to castration have been examined. In every case in which it is known that the original castration was incomplete the relations of the gonad have been precisely as postulated and diagrammed in b. On the other hand, in each of the five cases which showed delayed resumption of comb growth the condition has been either that shown in d or in e, precisely as postulated for gonad formation following total castration.

Comb growth in the fowl is a relatively sensitive indicator of endocrine function of the testis. In the absence of comb growth there is no testis tissue present in the animal, or such testis as is present is elaborating androgenic material at a sub-threshold level. In this connection the condition in animal 51 is interesting. At the time the regenerated testis was removed the comb had attained a size of 7.0 × 4.4 cm., and removal resulted in prompt regression of the comb. The mass excised weighed but 10 mg. When this minute mass of testis tissue produced comb growth of such magnitude it is by no means unreasonable to consider that no testis tissue is present in an animal which shows no comb growth over an extended period.

Upon histological examination testes known to have grown from retained fragments (fig. 2) are morphologically almost indistinguishable from normal bird testis or from autoplastic testis grafts (fig. 3). The tubules of these testes are uniformly large and present active spermatogenesis; very few or no immature tubules are seen. The testes from the five animals under consideration, on the other hand, differ strikingly from this pattern (figs. 4, 5, 6). There is an increased internal lobulation of these gonads with the lobules of varying size and irregular arrangement in contrast to the radial disposition and fairly uniform size of the lobules of the normal testis or hypertrophied fragment. The tubules are markedly smaller in diameter and are, with few exceptions, immature, with the degree of maturity ranging from a typical medullary cord through all the stages to an infrequently encountered completely mature tubule. Of these gonads those of one animal contain only sterile cords, those of two animals contain cords in which spermatogenesis has gone no further than spermatocytes; and those of two animals contain tubules in which spermatogenesis is in places complete.

If these testes do originate in the manner postulated, it does not necessarily follow that sex cord proliferation would be seen in sections. If, however, formation of sex cords from the peritoneum were seen in but one case, it would confirm the hypothesis regarding these gonads. In three of the five animals cords are quite obviously forming at the periphery and migrating into the substance of the gonad (fig. 7). Occasionally transitional stages between mesothelial cells of the serosa and larger, rounded cells under the serosa may be seen. In rarer instances it appears not impossible that fibroblasts of the tunica albuginea may be the cells of origin. In both circumstances the enlarged cells aggregate into cords and migrate inward, with the picture strikingly resembling that sometimes seen in the formation of cords in the ovarian cortex. As these cords grow deeper into the substance of the testis they increase in size and degree of development until ultimately mature spermatozoa may be found in certain of them.

## DISCUSSION

### *Gonad regeneration*

Regeneration of gonad following complete gonadectomy has been claimed by Davenport ('25), Parkes, Fielding and Brambell ('27), and Pallot ('28). Davenport reports that a very large number of his ovariectomized mice (64%) regenerated ovaries within a few weeks or months after their removal. His arguments for de novo formation of ovary in certain of his cases are the care with which the operation was done, many regenerated ovaries were not enclosed in a capsule, two ovaries regenerated in the place of one, and one case in which the regenerated ovary was found at a point 5 mm. distant from the site of the previous ovary. The originally excised ovaries were apparently not examined to ascertain their integrity, and the time of appearance of the regenerated ovary is uncertain.

The conclusion of Parkes, Fielding and Brambell is based on the following observations: of a large number of ovariectomized mice eleven showed resumption of oestrous cycles subsequent to an initial cessation of 12 to 113 days duration; serial section of the extirpated ovaries of six of these animals revealed that in five cases they had been removed intact; and ovarian tissue could be identified in these animals subsequent to operation.

The observations of Pallot are essentially the same as those of Parkes and his co-workers. He observed a return of oestrus in three of a series of rats shown by section of ovaries to have been totally ovariectomized. The non-oestrous phase was approximately 6 months in duration. In two of these animals ovary was found at autopsy.

These conclusions have not remained unchallenged. Hanson and Heys ('27) and Heys ('29, '31) have ovariectomized a large number of rats varying in age from 10 to 200 days at the time of operation. None of their animals under 40 days of age at operation showed any ovarian tissue subsequent to operation, a fact they attribute to the greater ease of complete extirpation in the young animal. In eight instances among the adults ovarian tissue was identified at autopsy, six of which had been completely ovariectomized as shown by serial section of the original ovaries. These authors entertain the opinion that ovarian regeneration occurs only following incomplete ovariectomy.

Haterius ('28) also denies de novo formation of the ovary following complete ovariectomy. His opinion is based largely, it seems, on the rarity of such apparent cases in his experiment (five out of ninety-six), although histological study revealed the original ovaries to be entire.

Pencharz ('29) insists that in neither rats nor mice does the ovary arise de novo, providing all ovarian substance has been removed. His conclusions are based on a study of the vaginal smears of completely and sub-totally ovariectomized animals. He observed an initial cessation of oestrous cycles, of variable duration, in partially ovariectomized animals.

The most serious flaw in Davenport's work is his failure to determine whether the ovaries had been removed intact. Another criticism lies in the fact that his only criterion of the presence of ovarian tissue is its detection at autopsy, which gives no indication of the time intervening between operation and regeneration or hypertrophy. The evidence of Parkes, Fielding, and Brambell and of Pallot is much more convincing, and Haterius' argument that histologic totality of the extirpated ovaries is not proof of completeness of removal is difficult to accept. Moreover, infrequency of regeneration seems to us by no means to constitute an argument for incompleteness of the original operation. The same remarks may be made concerning the views of Heys and Hanson. It would seem to make no difference how difficult the operation may be, the age of the animal, or the rarity of regeneration de novo; if the ovaries are removed entire, the animal is totally ovariectomized, and any ovarian tissue which may be observed subsequently cannot represent a hypertrophied fragment. In regard to the absence of oestrous phenomena, the commonly observed irregularities, and even absence of cycles, in unoperated animals constitute a more serious indictment of this criterion of absence of ovary than does the observation by Pencharz that the cessation and ultimate resumption of cycles in subtotally ovariectomized rats and mice is similar to that reported by Parkes and his collaborators in their animals. It seems reasonable that the attainment of threshold activity would present the same general picture, whether the minute ovary is growing from a fragment or from ovary formed de novo. There seems to be a widespread conviction that de novo formation of gonad is an impossibility. For example, Domm ('27) concludes that his birds were regenerating gonad from a fragment, despite his definite impression that all gonad tissue had been removed.

The relative freedom from cyclic activity on the part of the testis should make the male far more favorable than the female for a study of this nature; and the possession of an external character which responds quantitatively to testis hormone, and which reacts sharply to castration, should serve to render the male fowl an ideal test animal. The period of time during which no comb growth was exhibited by the animals in this report points rather definitely to absence of testis as its explanation. It is reasonably well established that a pale, capon comb is never seen in a fowl containing even a minute quantity of testis; it is reported by Benoit ('27) that 0.3 gm. of testis is sufficient to maintain the non-castrate condition, and 1 mg. of testis will produce growth of the comb. Greenwood and Crew ('27) in a study of 'developmental capons' found that the presence of 0.016 gm. of testis sufficed to produce and maintain vascularity of the comb. Our animal 51 exhibited a not inconsiderable growth of the comb associated with the presence of but 0.010 gm. of testis, the removal of which promptly produced regression and paling of the comb. A period of 6 months or more during which there is no comb growth can be satisfactorily explained only on the basis of total testis deficiency, especially since partial castration is followed, as is well known, by prompt and marked 'compensatory hypertrophy.'

It is unfortunate that not all the original testes removed from these animals were saved. However, those testes which were saved are intact and it is thus clear that at least three of the animals were totally castrated.

A striking evidence of de novo testis formation in these fowls is provided by the peritoneal relations. It is remarkable that the conditions postulated should have been met so precisely following the two types of operation. It is just possible, of course, that following incomplete removal of a testis the mesorchium might in some manner contract resulting in the establishment of an apparent retroperitoneal location of the fragment. Such an occurrence appears improbable, and the invariably pendant character of the numerous testes known to have developed from fragments decreases the likelihood of mesorchial retraction. On the contrary, it appears more likely that a de novo gonad could simulate superficially the relations of a fragment.

If it be accepted that these animals were totally gonadectomized, there are at least two possible sources of the new testis. The possibility of a minute, accessory gonad cannot be overlooked, although such a structure has not been reported in the fowl, and we have never seen accessory gonads in chick embryos despite examination of large numbers. The other, and postulated, possibility is proliferation of cords from the peritoneum. The finding of this particular occurrence in certain of our cases is a demonstration of de novo testis formation, even if the possibility of incompleteness of castration be admitted.

### *Germ-cell history*

While earlier writers considered the germinal epithelium the site of origin of the germ cells in the chick, the history given by Swift ('14, '15, '16) is the currently prevalent view. According to this account the primordial germ cells arise in the endoderm of the area pellucida during the primitive streak stage. Upon the extension of the mesoderm into this region the germ cells enter the mesoderm and migrate into the vascular channels. With the onset of circulation these cells are transported over the body in the blood stream, some of them arriving ultimately at the gonad-forming area where they leave the blood stream and enter the germinal epithelium. The cells so derived become the spermatogonia and presumably the definitive germ cells. This account has received confirmation from Woodger ('25) and Goldsmith ('28), with Goldsmith emphasizing this failure to observe any extensive degeneration of primordial germ cells and his belief that these germ cells are retained in an unbroken line to definitive spermatozoa and ova.

In addition to the foregoing purely descriptive studies the experimental approach has been applied to the germ cell problem in the chick. Reagan ('16) operated on chick embryos in pre-somite stages and succeeded in removing the crescent shaped area of the proamnion in which the germ cells are thought to arise. The gonads which formed in his operated embryos were completely sterile, showing only the presence of stroma with its peritoneal covering. Benoit ('30) subjected the germ cell area to the ultraviolet radiation and observed almost total destruction of the germ cells and only an infrequent germ cell in the gonads which formed. Dantschakoff, Dantschakoff and Bereskina ('31) have destroyed the germ cell crescent in part or entirely by cautery. Their results point to the cells of the crescent as the ancestors of the germ cells of the early gonad. Willier ('37) has removed the germ cell crescent of Swift and transplanted the remainder of the blastoderm to the chorio-allantoic membrane. Such gonads as developed were devoid of germ cells. The results of these experiments are in accord with the concept of an extra-gonadal origin of the primitive germ cells; but they obviously have no bearing on the equally fundamental question of the origin of definitive spermatozoa and ova. It is in connection with the latter problem that the results of Domm ('29) are of interest. This author found that if the left ovary is removed during the period when the primordial germ cells are still present in the rudimentary right ovary (as late as 3 weeks post-hatching, according to Brode, '28), the right ovary may transform into a testis containing male germ cells; if the same operation is performed upon chicks subsequent to the disappearance of the primordial germ cells in the right ovary, the latter becomes a sterile testis.

Numerous studies thus support the views of Swift on germ cell history in the fowl. Firket ('20), however, favors two generations of germ cells in the chick, the primordial germ cells which degenerate and a generation arising from the germinal epithelium which gives rise to the definitive germ cells. Moreover, in the adult fowl it has been reported by Fell ('23) and Gatenby ('24) that peritoneal cells may transform into germ cells. Gatenby's report deals with a single hen possessing an adenomatous and atrophic left ovary, anterior to which were found two small testicular masses. In these masses transformation of peritoneal cells into germ cells is described; however, none of the germ cells is advanced beyond the stage of the spermatogonium. Fell describes the gonads of eight hens in different stages of sex reversal which had been preceded by disease and atrophy of the ovary. The formation of testicular tissue was accomplished by a thickening of the germinal epithelium and a proliferation of sex cords which enlarged to form tubules. Three of the gonads contained mature spermatozoa while the remaining five were less mature.

The significance of our observations lies in their providing further instances of the origin of germ cells in the adult fowl from somatic cells, with the added feature that in our birds the process was not initiated by any demonstrable disease. Moreover, if we interpret our observations correctly, the germ cells in our specimens could have arisen only from somatic cells, and in so doing refute one of Weismann's original arguments.

These instances of the origin in the adult of germ cells from cells of the peritoneum are not construed as conflicting necessarily with an extra-gonadal origin of germ cells in this species; but they do demonstrate that there may be two seats of origin of germ cells, and that a transition of somatic cells into germ cells is possible in a species usually regarded as presenting an unbroken germ line.

## SUMMARY

Of 500 White Leghorn fowls castrated, five are considered from the following evidence to have regenerated testis subsequent to total bilateral gonadectomy:

1. From three of these five birds the original gonads were preserved and proved to be intact.

2. Comb growth was arrested in these five animals for a period varying from 6 months to 36 months following castration before being resumed, whereas following subtotal castrations comb growth was resumed within 1 month.

3. In one instance extensive comb growth after a prolonged arrest was produced by a regenerated testis weighing but

10 mg., an observation indicating that absence of comb growth is possible only when there is total absence of testis.

4. All of the regenerated testes were retroperitoneal in position, while hypertrophied fragments were always pendant in the body cavity.

5. The regenerated testes are readily distinguishable histologically from hypertrophied fragments which, in turn, resemble normal testis and autoplastic testis grafts.

6. In the regenerated testes cords may occasionally be seen forming from the peritoneum in a manner similar to that followed in the embryo.

Since the intermediate stages between mesothelial cells and mature spermatozoa have been seen in these regenerated testes it is concluded that somatic cells can transform into germ cells, this in a species whose definitive germ cells are generally regarded as arising only from primordial germ cells of endodermal origin.

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## PLATE 1

### EXPLANATION OF FIGURES

2 Testis growing from a fragment known to have been left at operation. × 70.

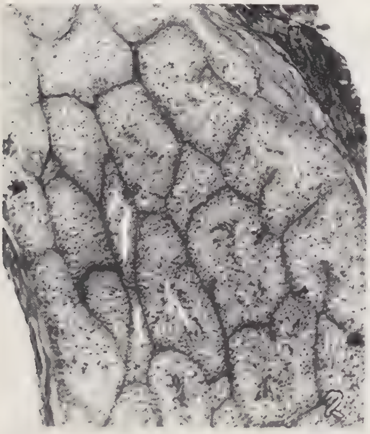
3 Autoplastic testis graft in juvenile bird. Residence 2 weeks. × 70.

4 Testis in bird 60 which showed no comb growth for 6 months following a castration recorded as complete. This gonad was retroperitoneal. Note immaturity of cords and increase of stroma. × 70.

5 Retroperitoneal gonad recovered from bird 38 which showed no growth of the comb until 29 months after a castration believed to have been complete. The tubules are immature. × 70.

6 Retroperitoneal gonad from bird 56 which showed no comb growth for 10 months following castration which was recorded as complete. The tubules are immature and the stroma is increased. × 70.

7 Area of gonad of bird 38 showing formation of cords from the peritoneum. × 210.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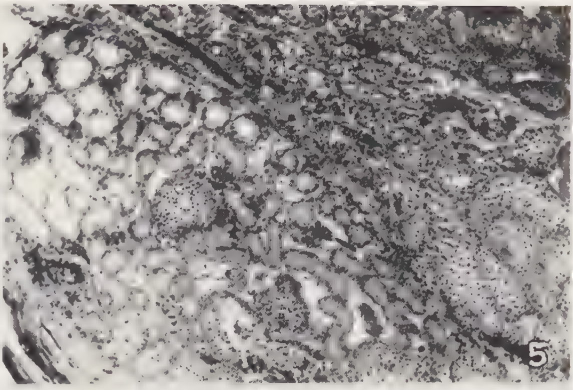
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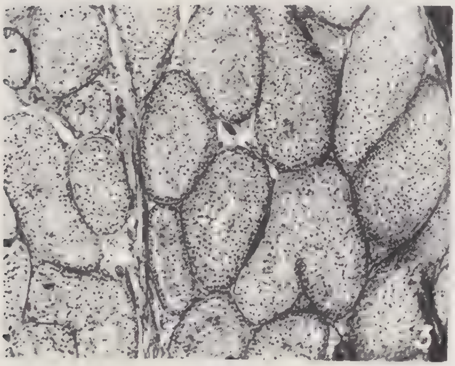
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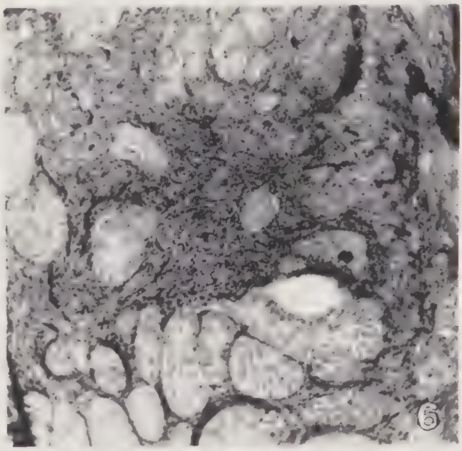
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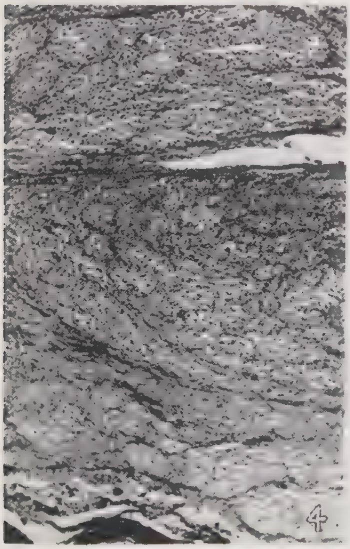
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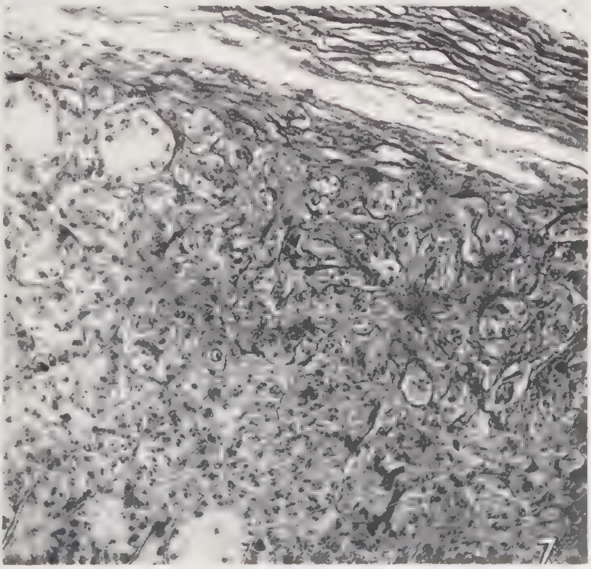
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# THE RELATIVE WEIGHT OF THE TESTES IN PRIMATES

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The relative size of the testes varies very strikingly among different primates. In order to gain some accurate information on this subject the author had collected some relevant data at different occasions during the past few years. Last year it became possible to increase this series of data to an extent resulting in definite conclusions. This opportunity arose during the Asiatic Primate Expedition of 1937 of which the author was a member.[1] Fifty-five of the adult male primates, recorded in the accompanying table, were weighed and autopsied on this expedition. Observations on seventeen other monkeys and apes (mostly from the Hopkins comparative-anatomical collection) have been added, as also data on three Negroes (unclaimed bodies of the anatomy department of the Johns Hopkins University). Corresponding data from the literature could be found only in a paper by Hrdlička ('25), who has listed the weights of the bodies and of the testes in seven adult howling monkeys from the field notes of the late Mr. J. L. Baer. The present paper records the relative weight of the testes in a total of eighty-two simian primates. The testes alone, without the epididymides, are considered here.

The first twelve primates listed in the table are platyrrhines or New World monkeys. All the others are catarrhines.

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[1] This expedition was organized by Mr. H. J. Coolidge, Jr. The Carnegie Institution of Washington generously contributed a grant toward the author's expenses on this expedition.

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*Weights of body and of testes and percentage relation between the two weights in eighty adult and two juvenile primates*

| SPECIES | CONDITION | BODY WEIGHT | TESTES WEIGHT | PERCENTAGE |
|---|---|---|---|---|
| | | kg. | gm. | |
| Oedipomidas geoffroyi | w. p. | 0.57 | 1.8 | 0.316 |
| Aotus zonalis | w. p. | 1.02 | 1.2 | 0.118 |
| Alouatta palliata | w. p. | 7.72 | 24.8 | 0.321 |
| Alouatta palliata [1] | w. f. | 6.92 | 30.1 | 0.435 |
| Alouatta palliata [1] | w. f. | 7.03 | 21.3 | 0.303 |
| Alouatta palliata [1] | w. f. | 7.03 | 24.8 | 0.352 |
| Alouatta palliata [1] | w. f. | 7.26 | 26.6 | 0.366 |
| Alouatta palliata [1] | w. f. | 7.26 | 19.5 | 0.268 |
| Alouatta palliata [1] | w. f. | 7.48 | 24.8 | 0.331 |
| Alouatta palliata [1] | w. f. | 7.82 | 28.3 | 0.362 |
| Average of 8 Alouatta | | *7.32* | *25.0* | *0.342* |
| Lagothrix lagothricha | c. p. | 5.22 | 11.2 | 0.215 |
| Ateles geoffroyi | w. p. | 7.94 | 13.4 | 0.169 |
| Macaca irus | w. f. | 3.63 | 19.6 | 0.540 |
| Macaca irus | w. f. | 3.85 | 18.9 | 0.491 |
| Macaca irus | w. f. | 3.85 | 17.8 | 0.463 |
| Macaca irus | w. f. | 4.30 | 27.9 | 0.648 |
| Macaca irus | w. f. | 4.30 | 36.0 | 0.837 |
| Macaca irus | w. f. | 4.54 | 37.8 | 0.833 |
| Macaca irus | w. f. | 4.54 | 27.4 | 0.604 |
| Macaca irus | w. f. | 4.76 | 43.8 | 0.920 |
| Macaca irus | w. f. | 4.98 | 26.9 | 0.540 |
| Macaca irus | w. f. | 4.98 | 36.4 | 0.731 |
| Macaca irus | w. f. | 5.44 | 36.8 | 0.676 |
| Macaca irus | w. f. | 5.44 | 39.7 | 0.730 |
| Average of 12 Macaca irus | | *4.55* | *30.8* | *0.668* |
| Macaca philippinensis | w. p. | 4.31 | 34.5 | 0.800 |
| Macaca philippinensis | w. p. | 4.53 | 36.0 | 0.794 |
| Macaca radiata | c. f. | 8.40 | 57.6 | 0.686 |
| Macaca nemestrinus | w. f. | 9.98 | 66.7 | 0.668 |
| Macaca mulatta | c. f. | 9.98 | 74.0 | 0.742 |
| Macaca mulatta | c. f. | 10.88 | 78.0 | 0.717 |
| Papio sphinx | c. p. | 31.98 | 88.9 | 0.278 |
| Pygathrix cristatus | c. p. | 4.77 | 2.8 | 0.059 |
| Pygathrix cristatus | w. f. | 4.98 | 0.6 | 0.012 |
| Pygathrix cristatus | w. f. | 5.67 | 5.0 | 0.088 |
| Pygathrix cristatus | w. f. | 5.90 | 6.6 | 0.112 |
| Pygathrix cristatus | w. f. | 5.90 | 4.8 | 0.081 |
| Pygathrix cristatus | w. f. | 6.12 | 5.4 | 0.088 |
| Pygathrix cristatus | w. f. | 6.35 | 6.3 | 0.099 |
| Pygathrix cristatus | w. f. | 6.58 | 7.0 | 0.106 |
| Pygathrix cristatus | w. f. | 6.58 | 5.5 | 0.084 |
| Pygathrix cristatus | w. f. | 6.58 | 7.8 | 0.118 |
| Pygathrix cristatus | w. f. | 7.03 | 5.9 | 0.084 |
| Pygathrix cristatus | w. f. | 7.48 | 6.7 | 0.089 |
| Pygathrix cristatus | w. f. | 7.48 | 6.1 | 0.081 |
| Pygathrix cristatus | w. f. | 7.94 | 7.0 | 0.088 |
| Average of 14 Pygathrix cris. | | *6.38* | *5.5* | *0.085* |

| SPECIES | CONDITION | BODY WEIGHT | TESTES WEIGHT | PERCENTAGE |
|---|---|---|---|---|
| | | *kg.* | *gm.* | |
| Pygathrix rubicundus | w. f. | 5.45 | 3.2 | 0.059 |
| Pygathrix rubicundus | w. f. | 5.67 | 3.0 | 0.053 |
| Pygathrix rubicundus | w. f. | 5.67 | 3.4 | 0.060 |
| Pygathrix rubicundus | w. f. | 6.12 | 3.6 | 0.059 |
| Pygathrix rubicundus | w. f. | 6.12 | 3.9 | 0.064 |
| Pygathrix rubicundus | w. f. | 6.12 | 3.4 | 0.056 |
| Pygathrix rubicundus | w. f. | 6.35 | 3.4 | 0.053 |
| Pygathrix rubicundus | w. f. | 6.35 | 3.2 | 0.050 |
| Pygathrix rubicundus | w. f. | 6.35 | 4.3 | 0.068 |
| Pygathrix rubicundus | w. f. | 6.58 | 4.9 | 0.074 |
| Pygathrix rubicundus | w. f. | 6.58 | 3.7 | 0.056 |
| Pygathrix rubicundus | w. f. | 7.03 | 3.7 | 0.053 |
| Average of 12 Pygathrix rubic. | | *6.20* | *3.6* | *0.059* |
| Nasalis larvatus | w. f. | 14.06 | 5.2 | 0.037 |
| Nasalis larvatus | w. f. | 20.42 | 7.2[2] | 0.035 |
| Nasalis larvatus | w. f. | 20.42 | 16.0 | 0.078 |
| Nasalis larvatus | w. f. | 20.42 | 11.8 | 0.058 |
| Nasalis larvatus | w. f. | 20.87 | 9.6 | 0.046 |
| Nasalis larvatus | w. f. | 20.87 | 14.6 | 0.070 |
| Nasalis larvatus | w. f. | 23.14 | 18.0 | 0.078 |
| Nasalis larvatus | w. f. | 23.59 | 13.2 | 0.056 |
| Average of 8 Nasalis larvat. | | *20.47* | *11.9* | *0.057* |
| Hylobates cinereus | w. p. | 4.98 | 2.9 | 0.058 |
| Hylobates cinereus | w. f. | 4.98 | 3.0 | 0.060 |
| Hylobates cinereus | w. f. | 4.98 | 6.1 | 0.122 |
| Hylobates cinereus | w. f. | 5.44 | 6.1 | 0.112 |
| Hylobates cinereus | w. f. | 5.90 | 5.4 | 0.091 |
| Hylobates cinereus | w. f. | 6.12 | 5.2 | 0.085 |
| Hylobates cinereus | w. f. | 6.35 | 3.7[2] | 0.058 |
| Average of 7 Hylobates ciner. | | *5.54* | *4.6* | *0.084* |
| Orang-utan[3] | w. f. | 29.95 | 29.0 | 0.097 |
| Orang-utan[3] | w. f. | 34.50 | 23.4 | 0.068 |
| Orang-utan | w. f. | 72.18 | 32.4 | 0.045 |
| Orang-utan | c. p. | 77.10 | 38.2 | 0.050 |
| Average of 2 adult orang-utan | | *74.64* | *35.3* | *0.048* |
| Chimpanzee | c. p. | 40.20 | 119.5 | 0.297 |
| Chimpanzee | c. p. | 43.77 | 108.0 | 0.247 |
| Chimpanzee | c. p. | 49.05 | 129.0 | 0.263 |
| Average of 3 chimpanzees | | *44.34* | *118.8* | *0.269* |
| Negro (37 years) | f. | 54.89 | 43.0 | 0.078 |
| Negro (41 years) | f. | 66.30 | 51.0 | 0.077 |
| Negro (31 years) | f. | 69.42 | 56.5 | 0.081 |
| Average of 3 Negroes | | *63.54* | *50.2* | *0.079* |

[1] After Hrdlička ('25).

[2] One testis undescended and vestigeal.

[3] Juvenile (C and M3 not yet erupted).

w., wild; c., captive; f., fresh; p., preserved (embalmed).

The macaques and baboons (Macaca and Papio) represent the subfamily Cercopithecinae and the langurs and proboscis monkeys (Pygathrix and Nasalis) the subfamily Semnopithecinae of the entire family of Old World monkeys. Last in the table are the higher primates: Gibbon (Hylobates), orang-utan, chimpanzee, and man. Unfortunately it was impossible to obtain actual weights on adult male gorillas. Based upon palpation of the scrotum in several preserved bodies of adult gorillas, the author does not hesitate to state that the testes of this anthropoid ape are comparatively small and appear to have a relative weight considerably below that in man.

The ratio of testicular weight to body weight amounts to more than 0.1% in all the New World monkeys examined. It is smallest in the night monkey (Aotus) and the spider monkey (Ateles) and largest in the marmoset (Oedipomidas) and the howling monkey (Alouatta). One of the latter was collected and embalmed by the writer in Nicaragua, the other seven come from Panama and were weighed fresh, yet their ratios appear fully comparable.

The relative weight of the testes differs to a very marked extent in the two subfamilies of Old World monkeys. In the five species of macaques the relative weight of the testes fluctuates beween 0.46 and 0.92 and the baboon has a corresponding value of 0.28. In langurs, on the other hand, the relative weight of the testes shows very much smaller averages, i.e., 0.08 in one species and 0.06 in another, the latter average occurring also in the closely allied proboscis monkey. Thus it is found that a rhesus monkey, weighing not quite 10 kg., has testes weighing 74 gm., whereas proboscis monkeys with an average body weight of over 20 kg. have an average testicular weight of only 11.9 gm.

Among the higher primates the chimpanzee has undoubtedly the proportionately heaviest testes. Adult orang-utans possess a smaller relative testicular weight than even the langurs. This relative weight averages about the same in gibbons as in man. The value for the latter agrees very closely with that given by Vierordt (1888, p. 17), who lists 0.08 as relative weight of the testes in adult man (presumably White). It is rather surprising to find that, e.g., a bonnet macaque weighing only 8.4 kg. has slightly heavier testes (57.6 gm.) than a Negro weighing 69.4 kg. whose testes tip the scale at 56.5 gm. In other words, per kilogram of body weight the former has 6.9 gm. of testicular tissue, whereas the latter only 0.8 gm. or eight and a half times less.

It is difficult to suggest any reasons for these wide discrepancies in the relative weight of the testes among different primates. They are not connected with any seasonal changes in the life of the monkeys; at least the author's field notes and the distribution of the ages of all the animals collected give no indication of any breeding season among the Old World primates. It was attempted to compare the relative size of the testes with the physiological age (as indicated by the state of attrition of the teeth) of the specimens in the series of macaques and of langurs, but it was soon apparent that there exists no correlation whatsoever between these two factors among adult animals.[2]

It seems possible, finally, that testes of widely differing relative size may contain the various tissues in very different proportions. Some of the testes, therefore, were sectioned and stained for microscopic study. The author is greatly indebted to his colleague, Dr. I. Gersh, for help in this undertaking. Sample sections of the testes were drawn thirty times enlarged with the camera lucida on heavy paper of uniform thickness. The parts representing (A) the tubules or the sex-cell producing glandular tissue were carefully cut out and these as well as the remaining parts, representing (B) the reticular and collagenous connective tissue, interstitial glandular tissue, blood vessels and nerves, were weighed separately with the following results:

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[2] It is interesting to note that the two juvenile orang-utans in the table possess relatively larger testes than the two full-grown orang-utans. On the other hand, small series of juvenile macaques and gibbons showed much smaller relative testicular weights than the corresponding series of adults.

| PRIMATE | RELATIVE TESTICULAR WEIGHT | A (TUBULES) | B (CONNECTIVE TISSUE) | A + B |
|---|---|---|---|---|
| | | *gm.* | *gm.* | *gm.* |
| Langur | 0.06 | 2.15 | 2.36 | 4.51 |
| Gibbon | 0.06 | 2.30 | 2.19 | 4.49 |
| Negro | 0.08 | 2.54 | 1.98 | 4.52 |
| Chimpanzee | 0.26 | 3.17 | 1.33 | 4.50 |
| Baboon | 0.28 | 3.31 | 1.18 | 4.49 |
| Macaque | 0.72 | 3.08 | 1.41 | 4.49 |

Contrary to what might have been expected, it is found that the primates with relatively large testes (chimpanzee, baboon and macaque) have a considerably greater proportion of sex-cell producing glandular tissue and a smaller proportion of connective tissue than have the primates (langur, gibbon and man) with comparatively small testes. On the assumption that the sample sections which have been analyzed are representative of the entire testes, it can be calculated that per kilogram of body weight the langur possesses 0.29 gm. of tubular tissue (A) and 0.31 gm. of connective tissue (B), whereas the macaque 4.9 gm. of tubular tissue and 2.3 gm. of connective tissue. This means that per unit of body weight the macaque has seventeen times more of the sex-cell producing glandular tissue than the langur.

Two of the adult, wild-shot primates in the table are marked as having one testis undescended and vestigeal. A few notes on this rare condition of unilateral cryptorchism are added here since it appears to have some connection with the size of the testes. In one of the adult proboscis monkeys only the right testis, weighing 7.0 gm., had descended in the scrotum and the left had been retained in the abdomen, weighing only 0.2 gm. This is unquestionably an abnormal condition and identical with the finding in an adult gibbon (Hylobates cinereus) from Borneo. In this ape the right testis was in the scrotum and the left way up in the abdomen; the former testis weighed 3.4 gm. and the latter only 0.3 gm. Among the male gibbons collected by the author in Asia there are two further cases of a very similar nature, though they appertain to juveniles. In a Hylobates cinereus, weighing 3.18 kg., in which the permanent canines and third molars had not yet erupted only the left testis of normal size was in the scrotum while the right, of exceedingly small size, was found in the abdomen. Exactly the same condition occurred in a Hylobates lar, weighing 3.40 kg. and with all permanent teeth, except the third molars. This unilateral cryptorchism was found in three out of sixty-two wild gibbons (twelve juveniles and fifty adults), or in 5% of the cases. The two cases in juveniles are not caused merely by a slightly retarded descent of one testis, as is evident from the vestigeal nature of the testis in the abdomen and also from the following observations on the normal time of descent of the testes in gibbons, based upon the author's field notes of last year:

| HYLOBATES | BODY WEIGHT | DENTITION | TESTES IN |
|---|---|---|---|
| | *kg.* | | |
| lar | 0.45 | Incomplete milk dentition | Scrotum |
| lar | 0.68 | Complete milk dentition | Abdomen |
| lar | 0.68 | Complete milk dentition | Scrotum |
| lar | 0.68 | Complete milk dentition | Scrotum |
| cinereus [1] | 0.91 | Complete milk dentition | Scrotum |
| lar | 1.36 | Incomplete permanent dentition | Abdomen |
| cinereus | 1.36 | Incomplete permanent dentition | Scrotum |
| cinereus [1] | 1.59 | Incomplete permanent dentition | Scrotum |
| lar | 2.04 | Incomplete permanent dentition | Scrotum |
| lar | 3.18 | Incomplete permanent dentition | Scrotum |
| cinereus | 3.40 | Incomplete permanent dentition | Scrotum |
| cinereus | 3.40 | Incomplete permanent dentition | Scrotum |
| lar | 3.63 | Incomplete permanent dentition | Scrotum |
| lar | 3.85 | Incomplete permanent dentition | Scrotum |
| lar | 4.08 | Incomplete permanent dentition | Scrotum |

NOTE: Adult male Hylobates lar have an average body weight of 5.7 kg. and adult male Hylobates cinereus one of 5.5 kg.

[1] These two specimens were living in captivity when examined, all the other gibbons listed are wild-shot specimens, examined fresh immediately after death.

In his paper on the genitalia of primates Wislocki ('36) has listed one juvenile Hylobates lar and two juvenile H. cinereus in all of which the testes had descended in the scrotum. It seems safe to conclude, therefore, that at least in these two species of gibbons the testes descend normally during infantile life, i.e., before any permanent teeth make their appearance.[3]

## SUMMARY

The weight of the body and of the testes and the percentage relation between the two is recorded for a total of eighty adult and two juvenile simian primates. This relative weight of the testes fluctuates between 0.1 and 0.4 among American monkeys. In monkeys of the Old World the testes are proportionately much heavier in the subfamily Cercopithecinae than in the subfamily Semnopithecinae. The relative testicular weight varies among five species of macaques between 0.46 and 0.92, whereas in langurs it averages only 0.06 and 0.08 in different series. Among higher primates this ratio amounts to 0.05 in orang-utans, 0.08 in gibbons and in man, and 0.27 in chimpanzees. Such striking differences in the relative size of the testes are best demonstrated by the example of a bonnet macaque, weighing 8 kg., which has slightly heavier testes than a Negro weighing 69 kg.

Small testes, relative to body size, contain a proportionately much smaller amount of tubules and a much larger amount of connective tissue than relatively large testes. It is calculated that, with equal body weight, a macaque, e.g., has seventeen times more sex-cell producing glandular tissue than has a langur.

In one proboscis monkey and in three gibbons there exists unilateral cryptorchism, i.e., one testis was retained in the abdomen and is of diminutive size and the other had descended in the scrotum and is of usual size. Normally the testes of these gibbons descend during infantile life.

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[3] The only juvenile Hylobates lar with both testes still in the abdomen has merely its first permanent molars besides the complete milk dentition and appears to be an exception, though Wislocki records undescended testes in three juvenile gibbons of other species (H. concolor and H. leucogenys).

# BOOK REVIEW

THE HYPOTHALAMUS: Morphological, Functional, Clinical and Surgical Aspects. By W. E. Le Gros Clark, D.Sc., F.R.C.S., F.R.S.; John Beattie, M.D., D.Sc.; George Riddoch, M.D., F.R.C.P. and Norman M. Dott, F.R.C.S. Ed. Published for the William Ramsay Henderson Trust by Oliver and Boyd, Edinburgh and London, 1938. xii + 211 pp. 12 s. 6 d.

This is a publication, in amplified form, of four lectures delivered at the University of Edinburgh during October, 1936, and hence is a general survey of the present status of the hypothalamic region of the brain.

The morphological part is credited to Le Gros Clark, who goes into a fair amount of detail on the nuclei and their fiber connections, together with a brief consideration of the development and blood supply. Excellent illustrations are used freely, there being over 100 in the book. After a terse review of the hypothalamus of lower vertebrates, followed by a consideration of the human brain, it is concluded that there is a progressive differentiation in this part of the central nervous system in the ascending scale. The relatively large hypothalamus in primitive forms is due rather to the small size of the forebrain than to any greater intrinsic importance of the hypothalamus in lower as compared with higher forms. The outstanding difference between the hypothalamus of lower mammals and man is the greater infiltration of the human hypothalamus with large cells.

The second section, by John Beattie, deals with the functional aspect and considers the role of the hypothalamus in various bodily reactions to heat and cold and the relationship of the hypothalamus to the hypophysis, 'sham rage' and sleep. Sleep is considered to be due to the damping-down of hypothalamic activities. After reviewing considerable physiological evidence, it is concluded that there are probably two general mechanisms. One responds co-ordinately to sympathetic reflexes and is more posteriorly located. The other is connected with the parasympathetic reflexes. In a later clinical section the diffuse arrangement of the first division is stressed while in the surgical section of the book there is a strong tendency to recognize well-defined functional centers, though in close proximity to each other. The specific function of a few of the nuclei is suggested such as the regulation of the production of an anti-diuretic hormone by the supra-optic nucleus and the control of the liberation of the diabetogenic hormone by the ventromedial nucleus.

The clinical phase is written by George Riddoch who discusses polyuria, polydipsia, bulimia, glycosuria, obesity, cachexia, sexual disorders, abnormal sleep, failure in temperature regulation, modification of the blood picture, ulceration of the alimentary tract, diencephalic epilepsy and emotional disorders in connection with lesions in the region of the third ventricle. Clinical evidence from such sources support the main thesis, namely, that the hypothalamus is the main subcortical integrating mechanism of the autonomic nervous system.

Norman M. Dott contributes the final section, dealing with the surgical side by means of case reports on a series of epidermoid hypophyseal tumors with notes on the operative procedure.

The tone of the book is optimistic and should encourage all who are working with the hypothalamic region. The style is clear and the workmanship good. There is both an author and a subject index. A list of 300 selected references is included.

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## BOOK REVIEW

METHODS OF TISSUE CULTURE. By Raymond C. Parker, Ph.D., Associate in Experimental Surgery, The Rockefeller Institute for Medical Research, New York. Price, $5.00. 292 pp.; 109 illustrations. Paul B. Hoeber, Inc., publisher, New York, 1938.

In this book Doctor Parker describes the basic tissue culture procedures currently in use at The Rockefeller Institute. The excellent manner in which this is done reflects his intimate personal experience with the subject matter. In his brief but excellent historical survey he points out that the original technique of Harrison has been modified and improved by a number of workers. He makes no attempt to describe in detail modifications not in use in the Carrel Institute.

The author has given an exceptionally complete description of the techniques. The book is very well illustrated. A valuable feature is his reference to sources of unusual materials and instruments.

A laboratory plan is presented. Necessary instruments and glassware are listed. There is a chapter on cleansing and sterilization procedures.

The author then describes the basic techniques such as the preparation of natural and artificial media, testing pH, and osmotic pressure, as well as the planting of flask and slide cultures.

The chapter on growth measurements and their interpretation is brief but adequate. The necessary references to original papers are provided.

The chapter on photomicrography and microcinematography contains a very practical treatment of these subjects which should prove useful.

Of special interest is the chapter on "Some Applications of Tissue Culture to Experimental Biology and Medicine." Here the author gives a brief resume of work done with tissue culture technique in experimental cytology, the study of tumors, viruses, hypersensitivity and immunity. This chapter will serve exceptionally well in orienting new workers in this field.

This book is essentially a manual on technique and as such should prove very popular. It contains just enough historical survey and treatment of results for purposes of orientation. The author's good judgment is reflected in the fact that he does not attempt to cover all the work which has been done with tissue culture technique. The field has become so large that this is no longer feasible.

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# THE FOSSA OVALIS, AND RELATED BLOOD VESSELS[1]

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SIXTEEN FIGURES

The descriptions of the fossa ovalis presented herein are based upon permanent records obtained consecutively from dissections of 200 thighs. Eight specimens were females, 192 males; sixteen were from one side of as many subjects, 184 from both thighs of ninety-two subjects. The illustrations (figs. 1 to 16) represent the anatomy of selected fossae, from male bodies, arranged in the order of increasing size of the fossa. In 100 cases the relationship of femoral and vein to the fossa was recorded.

## FOSSA OVALIS

The fossa ovalis (O.T. saphenous opening) is regularly described as possessing a sharp lateral edge of falciform contour, an ill-defined medial edge, and concave, proximal and distal margins or cornua. Its dimensions are said to be variable, although the degree of variability is not recorded.

In the present study the occurrence of the typical form predominates (figs. 10 to 13). But not infrequently, in small fossae, the medial edge is well-defined, the inferior cornu continued proximally to closely approach, or actually reach, the superior cornu (figs. 1 to 8), thus forming a complete oval; rarely the opening resembles an enlarged hiatus rather than a true fossa (fig. 1). When thus reduced in size, the

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[1] Contribution no. 276 from the Anatomical Laboratories of Northwestern University Medical School.

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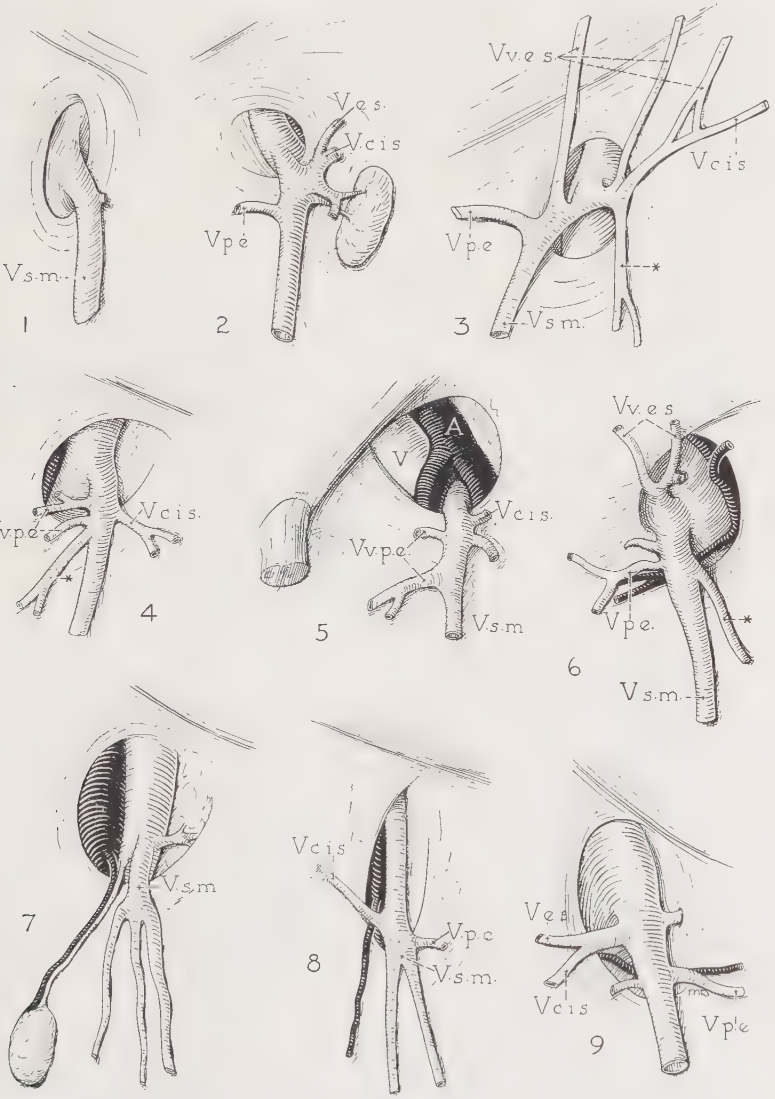
medial and lateral margins are not situated at different dorsoventral levels; the medial crus, spreading toward the lateral one, covers completely the declivity between the subjacent iliopsoas and pectineus muscles, the margins of the fascia clasping the confluens of saphenous and femoral veins.

In outline, the fossa is occasionally almost circular (figs. 2 and 7). Usually, however, it is oval (figs. 1, 4, 8 to 13); when thus compressed laterally, the long axis may be placed vertically (figs. 8, 10 and 12), or obliquely (figs. 4, 11 and 15); the narrowness may be strikingly displayed (figs. 8 and 10) or poorly (figs. 13 and 15). Occasionally the fossa is dart-shaped, with the pointed end directed downward (figs. 14 and 16).

The relationship of the superior crus to the inguinal ligament is inconstant. It is conventionally described as being attached above to the medial half of the inguinal ligament. When the fossa is proximally placed in the thigh, the cornu appears as a derivative of the inguinal ligament (figs. 5, 6 and 10), the ligament itself seeming to serve as one boundary of the fossa. Its independence in other instances is equally striking, an appreciable distance often intervening between the ligament and the cornu (fig. 2, 2 cm.; fig. 3 and 15, 1.5 cm.; figs. 14 and 16, 0.5 cm.).

Within the space of the fossa proper accessory hiatuses not infrequently occur, transmitting muscular tributaries to the larger veins (see under Vessels hereinafter). These appear in simple form (figs. 9, 12 and 14) or as multiple orifices (fig. 13). Auxiliary fossae occur near the major one (figs. 10 and 13).

In size the greatest variability is expressed (table 1). The smallest fossa in the 200 is 1.6 cm. in length, 1.7 cm. in width (fig. 2); the narrowest measures 1.0 cm. (fig. 1), the shortest 1.6 cm. (fig. 2). The largest fossa in the group is 8.5 cm. in length, 3.5 cm. in width (fig. 16); the widest measures 6.4 cm., the longest 8.5 cm. (fig. 16). Ninety per cent of the specimens (i.e., 180 fossae) measure between 3.0 cm. (fig. 5) and 6.4 cm. in length (fig. 15); 53.5% (107 fossae) measure between 3.5 cm. (fig. 6) and 5.4 cm. (fig. 11). The average length is 4.6 cm. In width, 87% (174 fossae) are between 1.5 cm. and 3.9 cm.; 70.5% (141 fossae) are between 2.0 cm. (fig. 10) and 3.9 cm. The average width is 2.8 cm.



Figs. 1 to 9 Fossa ovalis, with related blood vessels one-half natural size. Abbreviations: V.s.m., vena saphena magna; V.e.s., vena epigastrica superficialis; V.c.i.s., vena circumflex ilium superficialis; V.p.e., vena pudenda externa. Asterisk indicates accessory saphenous tributary.

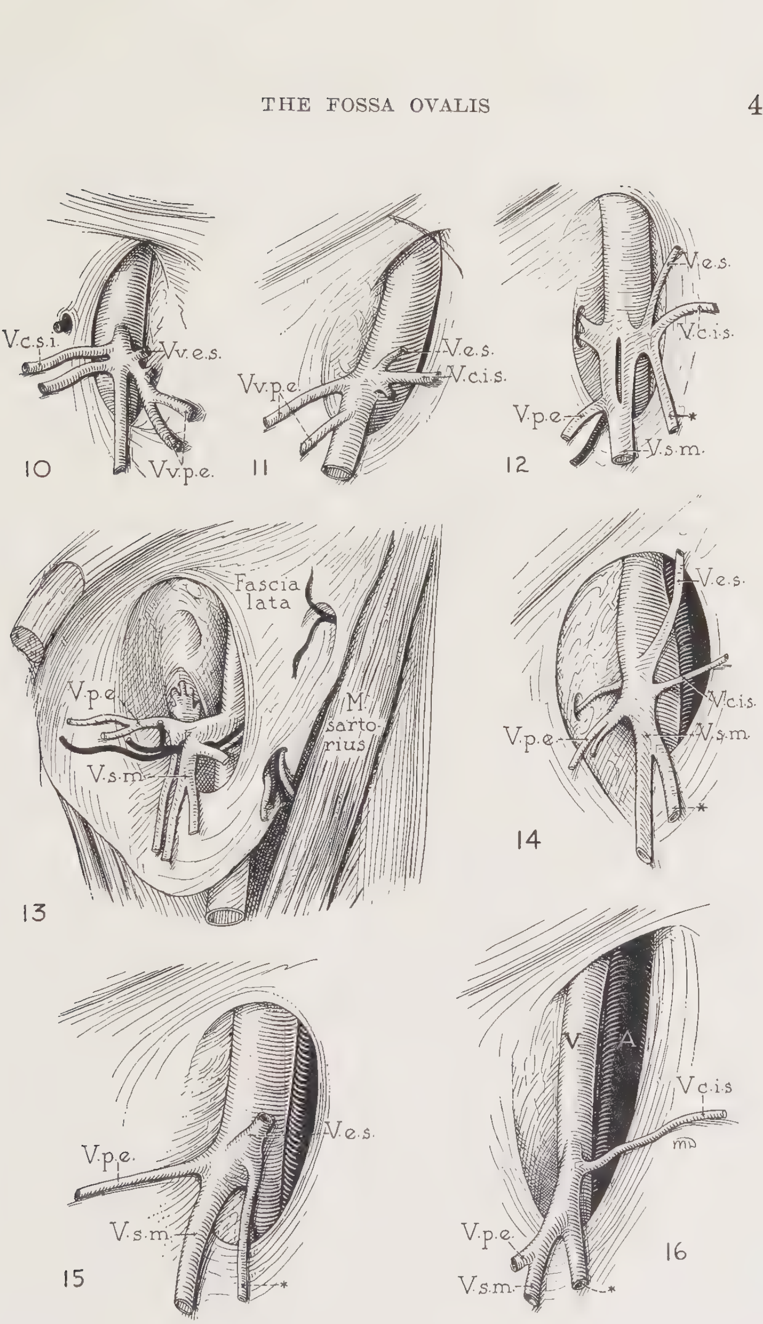
Without exception fossae are capacious for the vessels which they transmit; in only one case does the circumference of the saphenous vein approach that of the fossa through which it passes (fig. 1); regularly a very considerable space, unoccupied by vessels, is included between the cornua of the fossa (e.g., figs. 10 to 16), the size of contents in no way affecting the proportions of the orifice. So ample, in fact, is the space of the fossa, that the contained femoral vein is almost invariably exposed throughout its width. Moreover, in 83% of 100 consecutive cases the femoral artery, also, is wholly or partially exposed. More specificially, in 36%, three-fourths to all of its breadth is in view (figs. 7 and 16); in 30%, one-half to one-fourth; in 17%, not only is the artery entirely in view, but space occurs laterally, unoccupied by vessel (fig. 5).

TABLE 1

*Dimensions of fossa ovalis*

| *Length in centimeters* | *Number of specimens* | *Width in centimeters* | *Number of specimens* |
|---|---|---|---|
| 1.6 | 1 | 1.0–1.4 | 11 |
| 2.0–2.4 | 4 | 1.5–1.9 | 19 |
| 2.5–2.9 | 6 | 2.0–2.4 | 48 |
| 3.0–3.4 | 19 | 2.5–2.9 | 47 |
| 3.5–3.9 | 21 | 3.0–3.4 | 46 |
| 4.0–4.4 | 38 | 3.5–3.9 | 14 |
| 4.5–4.9 | 35 | 4.0–4.4 | 7 |
| 5.0–5.4 | 34 | 4.5–4.9 | 3 |
| 5.5–5.9 | 21 | 5.0–5.4 | 3 |
| 6.0–6.4 | 12 | 5.5–5.9 | 0 |
| 6.5–6.9 | 4 | 6.0–6.4 | 2 |
| 7.0–7.4 | 3 | | |
| 7.5 | 1 | | |
| 8.5 | 1 | | |
| | 200 | | 200 |

There is apparently no constant relationship between the size of the body and the caliber of the fossa. In specimens of the same height the fossae may be of widely different proportions, fossae of great size (fig. 16) and small (fig. 1) occurring in specimens of the same height (69 inches), others



Figs. 10 to 16 Fossa ovalis and vessels, continued. One-half natural size. Figure 10 is of the right side (as are figs. 1, 4, 7 to 9); all others are from the left side.

of similar proportions (figs. 9 and 10) appearing in cadavers of differing heights (64 and 70 inches, respectively).

## BLOOD VESSELS[2]

The veins of the scrotal, inguinal, and anterior femoral regions are usually received by the great saphenous tributary of the femoral vein, in the immediate neighborhood of the fossa ovalis. In many of the cases, the superficial epigastric and superficial iliac circumflex veins enter the saphenous vein as independent channels (figs. 10, 14). Any of the three major vessels may be doubled (figs. 4 and 5, Vv.p.e.; fig. 6, Vv.e.s.); two adjacent tributaries may be confluent (figs. 2, 9, 11 and 12, V.e.s. and V.c.i.s.; fig. 3, V.e.s. and V.p.e.). Not infrequently veins are received from the femoral muscles through accessory hiatuses in the deep fascia (figs. 12 to 14), and from lymphatic glands lodged in the superficial fascia (figs. 2 and 7). In some instances the arrangement of superficial veins from the area proximal to the fossa ovalis is excessively simple (figs. 1 and 7), while, in other cases the venous pattern is complex. In one instance (fig. 3) seven channels, in two groups (medial and lateral) converge upon a short vessel tributary to the femoral vein: a saphenous vein joins a superficial external pudendal to form the common medial channel, the latter receiving, superiorly, one of three superficial epigastric veins; the most laterally placed of the superficial epigastric veins, joining the superficial iliac circumflex, forms the lateral venous channel, which near the confluens with the medial channel, receives a third superficial epigastric vein superiorly, a second saphenous vein inferiorly. A vein may be bifid through part of its course (fig. 12); accessory saphenous veins are common (figs. 3, 4, 6, 12 to 16), occasionally as many as three occurring within the territory of the femoral triangle (fig. 7).

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[2] Only those cases which are illustrated will be considered here. A detailed study of the vessels of the lower abdominal wall and of the thigh is now in progress; the results of this study will be reported in a subsequent paper.

# THE PYRAMIDALIS MUSCLE [1]

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FIVE FIGURES

The present study is based upon the consecutive examination of 165 dissection-room subjects, of which 155 are males, 10 females. The measurement of height is taken from the pubic crest to the tip of the apex of the muscle along the latter's medial margin; that of width is taken transversely at the pubic crest.

*Form and structure.* In form and structure the muscle may exhibit occasional departure from the typical. The following aberrations were observed; one example of a muscle of quadrilateral rather than pyramidal form, its inferior third aponeurotic (fig. 3); one, of a muscle triangular in outline and occupying the expected position but almost entirely aponeurotic in structure—a very thin tendinous band containing only a few indistinct muscle fibers (fig. 4). The latter muscle would seem to be an extreme example of those pyramidales which Chudzinski (1898) described as being predominantly tendinous. In all other instances in our series the muscle, whether elongate or excessively short, assumed the regular triangular outline and consisted principally of fleshy body.

*Course and attachments.* By most authorities it is stated that the muscle arises from the anterior surface of the superior ramus, and symphysis, of the pubis. According to an occasional description, however, the origin occurs on the upper border of the bone. In the experience of the writers, the origin is always an anterior, not a superior, one. In fact, the origin may extend inferiorly upon the ramus of the pubis for a distance of 0.5 cm. to 2.0 cm.; in eighty-five specimens of muscle measured, the inferior extension was 0.5 cm. to 0.9 cm. in seventeen, 1.0 cm. to 1.5 cm. in fifty, 1.6 cm. to 2.0 cm. in eighteen.

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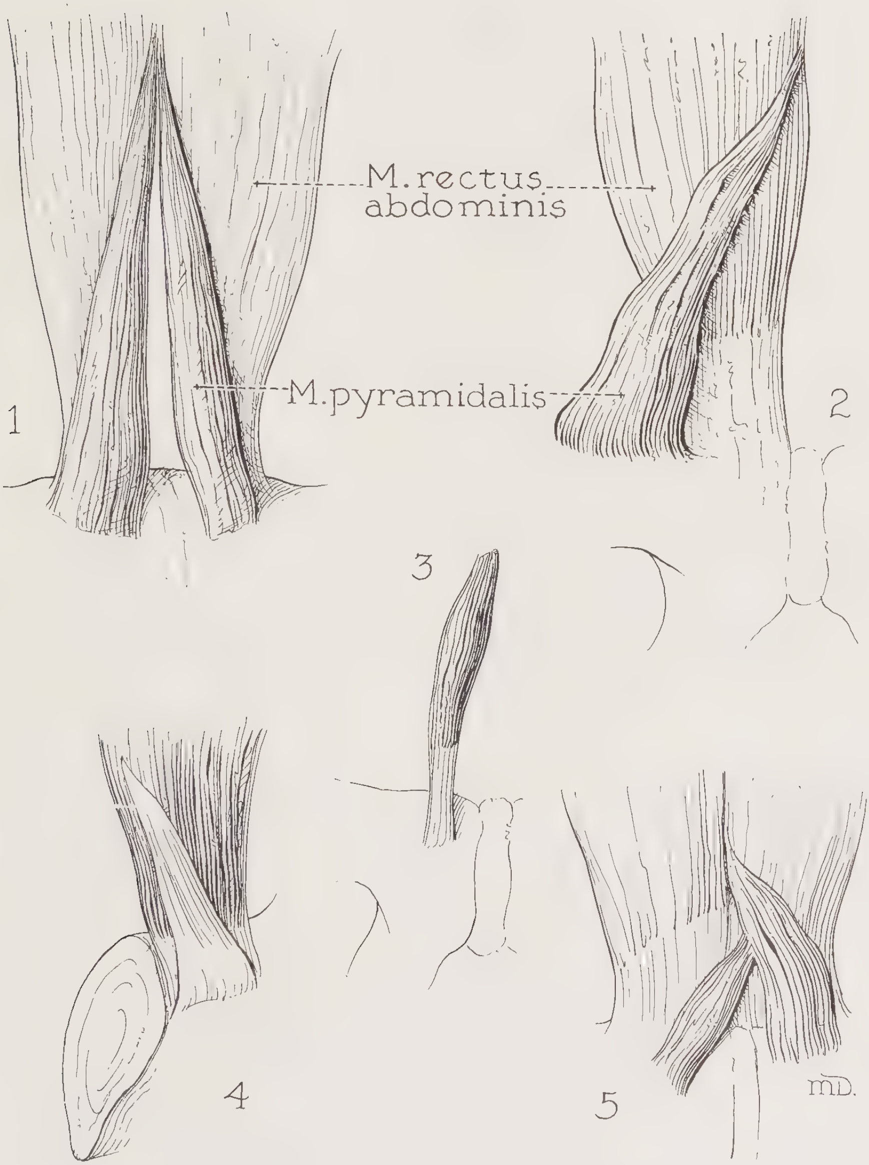
[1] Contribution no. 278 from the Anatomical Laboratories of Northwestern University Medical School.

The course of the muscle is almost invariably vertical, the medial margin lying close to the midline, and the aponeurotic fibers of insertion interdigitating, to some extent, at the linea alba. Two exceptions to this arrangement were noted in our set of specimens: one muscle, the base of which was removed laterally from the symphysis so as to expose the origin of the rectus abdominis muscle, crossed the latter in its course (fig. 2); another, arising some distance laterad to the symphysis, crossed the midline and was inserted into the deep surface of the normal pyramidalis of the opposite side (fig. 5).

*Size.* Anatomists have long noted variability in the degree of development of the pyramidalis, but actual measurements of individual muscles are rare in the literature. We have observed a specimen 12.0 cm. long, measured from the pubic crest (13.5 cm., from the point of origin), others as short as 1.5 cm., which consisted of merely a few fascicles, so small, in one case, as to extend barely above the pubic crest.

Of the 295 muscles studied by the writers 269 (91%) are between 3.0 cm. and 9.9 cm. in length, 157 (53%) between 5.0 cm. and 7.9 cm. (table 1). Two hundred seventy-one (92%) are between 1.0 cm. and 1.9 cm. in width. The average length is 6.82 cm., the average width 1.98 cm. (see table 2 for comparison with figures of other authors).

Using as our basis fifty consecutive cadavers, it is found that there is no relationship between length of muscle and height of body: long muscles may be found in short persons or short muscles in tall individuals. This observation confirms that of Vallois ('26) who could find no relationship between size of the muscle and size of pelvis or size of abdomen. In regard to disparity in size of members of pairs, it may be said that out of 100 muscles (fifty bodies), twenty bodies show



Figs. 1 to 5 Specimens of pyramidalis muscle. Two-thirds natural size. Figure 1, long muscles of normal form; figures 2 to 5, muscles of aberrant form, attachment or structure.

TABLE 1

*Pyramidalis*

| *Length*[1] |  | *Width*[2] |  |
|---|---|---|---|
| Cm. | No. | Cm. | No. |
| 1.5– 1.9 | 5 | 0.5–0.9 | 7 |
| 2.0– 2.9 | 6 | 1.0–1.9 | 172 |
| 3.0– 3.9 | 21 | 2.0–2.9 | 99 |
| 4.0– 4.9 | 39 | 3.0–3.9 | 10 |
| 5.0– 5.9 | 54 | 4.0–4.9 | 3 |
| 6.0– 6.9 | 45 | 5.0–5.9 | 0 |
| 7.0– 7.9 | 58 | 6.0 | 4 |
| 8.0– 8.9 | 30 |  |  |
| 9.0– 9.9 | 22 |  |  |
| 10.0–10.9 | 11 |  |  |
| 11.0–11.9 | 3 |  |  |
| 12.0 | 1 |  |  |
|  | 295 |  | 295 |

[1] It may be of service to record less inclusive groups, of 0.5 cm. each. These are as follows: 1.5–1.9 cm., 5 specimens; 2.0–2.4, 2; 2.5–2.9, 4; 3.0–3.4, 11; 3.5–3.9, 10; 4.0–4.4, 19; 4.5–4.9, 20; 5.0–5.4, 36; 5.5–5.9, 18; 6.0–6.4, 29; 6.5–6.9, 16; 7.0–7.4, 39; 7.5–7.9, 19; 8.0–8.4, 18; 8.5–8.9, 12; 9.0–9.4, 16; 9.5–9.9, 6; 10.0–10.4, 7; 10.5–10.9, 4; 11.0–11.4, 2; 11.5–11.9, 1; 12.0, 1.

[2] Of these, the less inclusive categories are as follows: 0.5–0.9 cm., 7; 1.0–1.4, 91; 1.5–1.9, 81; 2.0–2.4, 81; 2.5–2.9, 18; 3.0–3.4, 9; 3.5–3.9, 1 (of 3.5); 4.0–44, 3 (of 4.0 cm. only); 4.5–4.9, 0; 5.0–5.4, 0; 5.5–5.9, 0; 6.0, 4.

TABLE 2

*Pyramidalis*

A. Length

| *Author* | *Average length, cm.* | *Maximum, cm.* | *Minimum, cm.* |
|---|---|---|---|
| Chudzinski (1898) | 6.2 | 9.2 | 3.9 |
| Loth ('19) | 6.2 | 13.8 | 2.0 |
| Vallois ('26) | 7.17 | 10.0 | 3.0 |
| Nakamura ('35) | 6.77 | 10.3 | 5.7 |
| Anson et al. ('38) | 6.82 | 12.0 | 1.5 |

B. Width

| *Author* | *Average width, cm.* | *Maximum, cm.* | *Minimum, cm.* |
|---|---|---|---|
| Crudzinski (1898) | 2.35 | 3.7 | 2.2 |
| Loth ('19) | 1.60 | 3.0 | 0.2 |
| Vallois ('26) | 2.03 | . . . | . . |
| Nakamura ('35) | 1.92 | 3.0 | 1.0 |
| Anson et al. ('38) | 1.98 | 6.0 | 0.5 |

muscles of the same or approximately the same height (i.e., within 2 mm. of exact correspondence); in fifteen bodies the muscle of the right side is longer, in fifteen that of the left. This is in direct contradiction to the statement of Hallett (1848) that one muscle, usually the right, is almost always longer than its mate. Where disparate (thirty bodies) six are between one-sixteenth to one-tenth longer, fifteen are between one-ninth and one-fifth, seven between one-fourth and one-half. In one specimen the longer is one and two-thirds times the length of the shorter, in another, the muscle of one side merely reaches the level of the pubic crest, while its companion is 3 cm. long.

TABLE 3

*Absence of pyramidalis*

| *Investigator* | *Sides examined* | *Sides absent* | *Per cent absent* |
|---|---|---|---|
| Schwalbe and Pfitzner (1889, 1891) | 393 | 50 | 12.7 |
| Dwight[1] (1893) | 673 | 141 | 21.0 |
| LeDouble (1897) | 486 | 43 | 8.8 |
| Chudzinski (1898) | 36 | 0 | 0.0 |
| Koganei, Arai and Shikinami ('03) | 192 | 6 | 3.1 |
| Adachi ('09) | 314 | 12 | 3.8 |
| Wagenseil ('09) | 80 | 1 | 1.2 |
| Nakano ('23) | 40 | 0 | 0.0 |
| Vallois ('26) | 118 | 20 | 17.7 |
| Ancel (cited by Vallois) | 196 | 53 | 27.2 |
| Loth ('31) | 506 | 84 | 16.6 |
| Nakamura ('35) | 120 | 4 | 3.3 |
| Anson et al. ('38) | 330 | 35 | 10.6 |
| | 3484 | 449 | 12.8 |

The totals for other investigators, without the addition of our figures, are 3154 sides examined, 414 sides absent: a percentage absence of 13.1%.

[1] Dwight's second series of 250 bodies is not included in this table, because it is not clear from his article whether any or all of these bodies were included in his first series.

*Supernumerary muscles.* The existence of two or more muscles on one side has been observed by several authors. Winslow (1776) noted that there may be two muscles on one side, one on the other; this observation has been repeated by Cruveilhier (1854) and others. LeDouble (1897) quotes

Sabatier and Poland as having seen the muscle doubled on both sides, and Hallett (1848) saw one such specimen. Horner (1840) states that sometimes two, three or four muscles are seen on a side. According to Chudzinski (1898), the muscle, when unilateral, may double itself on the side where present. In our study one case of doubling, none of trebling or quadrupling, was observed: in the former, the muscle is bilaminar, the strata fused at the extremeties.

*Occurrence.* In our series, the muscle was found on 295 out of 330 sides, a percentage absence of 10.6%. Table 3 gives the findings of other investigators; this tabulation includes all the data available in the literature with the exception of that of Hallett (1848, 1849), the earliest investigator, whose figures are incomplete and inexact.[2]

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# THE EFFECTS OF CONTINUOUS INTRAVENOUS INJECTION OF AN EXTRACT OF THE ALPHA CELLS OF THE GUINEA PIG PANCREAS ON THE INTACT GUINEA PIG

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TWO PLATES (FOURTEEN FIGURES)

From the studies of the solubilities of the secretion granules of the guinea pig pancreas, reported in an earlier paper (S. H. Bensley, '38) it was apparent that the granules of the alpha cells of the islands of Langerhans were removed by distilled water, whereas the beta granules and the zymogen granules were not. The latter were removed by 70% alcohol while the alpha granules were not. However, the alpha granules preserved with alcohol could be subsequently removed by water. On the basis of these findings an extract of the pancreas, designed to contain the substance of the alpha cell granules, was made in the following way.

The complete pancreas was removed from three freshly killed guinea pigs which had just been fed. The combined pancreases weighed about 5 gm. These were ground up thoroughly with sand, by means of mortar and pestle, in 75 cc. of distilled water and the whole was centrifuged at high speed until the supernatant fluid was only slightly opalescent, to remove the beta and zymogen granules. The supernatant fat was skimmed off and the fluid was then carefully decanted and evaporated in an incubator at about 40°C.

The dried residue was extracted with absolute ether to remove any fat or fatty acid and the whole centrifuged. The ether was then decanted and the sediment dried in a vacuum desiccator. Subsequently the residue was treated with 75 cc. of distilled water and stirred well until the particles were in suspension. This suspension was then centrifuged at high speeds and the supernatant fluid decanted, filtered through a sterilized, washed Berkefeld filter and collected into a sterile flask. The sterile extract was transferred to a sterile bottle which was connected to the apparatus for continuous intravenous injection, as described by Woerner ('38).

In the process of making and testing such an extract certain difficulties were encountered and the following precautions were found necessary. Recently fed animals were preferred for obtaining pancreases for extraction because under such conditions the lipase content of the pancreas appears to be at a minimum. Neutral fats are more readily removed from the centrifuged extract. Care was taken not to nick the intestine in removing the pancreas. Contamination with intestinal juice seems to increase lipolysis and change the pH of the extract. Contamination with alkali was avoided to prevent zymogen and beta granules from going into solution in the extract. Extraction with ether was introduced because the neutral facts proved to be of a low melting point and after heat of the centrifuge or on a hot day, it was impossible to remove all the fat by skimming after centrifugation. Rapid evaporation of the aqueous extract without heat resulted in a product which gave the most pronounced results. But this was impossible on humid days. Heat seemed to reduce the potency of the extract. The aqueous extract did not keep well on standing, even at refrigeration temperatures, and best results were obtained with freshly prepared extracts.

Chemical tests of the extract showed a pH of about 7.0, a faint Millon reaction and the absence of fatty acids.

The following experiments were undertaken:

1. A male guinea pig was weighed and prepared for intravenous injection according to the method described by Woerner ('38), and connected to the apparatus. During a period of recovery (from 12 to 24 hours) normal saline solution was injected at the rate of 2 cc. per hour. The alpha cell

extract was then substituted for 24 hours at the same rate. The animal was then disconnected from the apparatus, weighed, and killed by bleeding. Pieces of the liver and pancreas were immediately fixed in acetic-osmic bichromate, formalin-chrome sublimate, and formalin-alcohol, for cytological studies. Smears of fresh liver and pancreas were treated with a drop of 2% osmic acid and examined directly. This experiment was repeated on four animals.

2. A male guinea pig, weighed and prepared for intravenous injection, received normal salt solution at the rate of 2 cc. per hour for 24 hours, then dextrose at the rate of 2 gm. per kilogram of body weight per hour for 24 hours, then the same amount of dextrose plus alpha cell extract at the rate of 2 cc. per hour for 3 days. The animal was then weighed, killed by bleeding and the liver and pancreas immediately fixed for cytological studies.

3. A male guinea pig was weighed, fasted for 24 hours, weighed, fed for 24 hours, weighed again and prepared for intravenous injection. At this time a biopsy of the liver was taken and the tissue fixed in acetic-osmic-bichromate. Normal salt solution was injected at a rate of 2 cc. per hour for 24 hours. Alpha cell extract was then substituted at the same rate for 24 hours, at the end of which time another biopsy of the liver was made and the tissue treated in the same way as the first. Alpha cell extract at the same rate plus dextrose at the rate of 2+ gm. per kilogram of body weight per hour were injected for the next 24 hours. The animal was then weighed, killed by bleeding, and pieces of the liver and pancreas were fixed immediately for cytological studies.

4. Studies on fasted animals as controls were made on two series: a) two older guinea pigs, weighing over 500 gm. Four fed animals in this group showed considerable fat in the livers. b) Two younger guinea pigs, weighing between 300 and 400 gm. Five fed animals in this group showed practically no fat in the livers. (Experiment 1 was made on animals from group a; experiment 3 on an animal from group b.)

The animals were weighed, fasted for 24 to 48 hours, weighed again, killed by bleeding and the tissues fixed immediately for cytological studies.

Pieces of tissue fixed in acetic-osmic-bichromate for 24 hours were embedded in paraffin, sectioned at 4 μ, studied for fat distribution or stained with aniline acid fuchsin and methyl green for mitochondrial studies. Although smears of the liver, stained with colloidal Sudan III indicated that the osmic-combining fat did not represent all the globule fat in the liver cells, nevertheless the acetic-osmic-bichromate fixation was considered adequate for comparative studies. Pieces of pancreas fixed in formalin-chrome sublimate were embedded in paraffin, sectioned at 4 μ, stained in neutral gentian for studies of the granule content of the alpha and beta cells of the islands of Langerhans, and of the acinar cells. Pieces of liver fixed in formalin-alcohol were embedded in paraffin, sectioned at 4 μ, stained with Best's carmine or with Bauer-Feulgen stain for studies of glycogen content.

Before the microscopic findings in these tissues are described a few observations may be recorded. In the animals injected with alpha extract there was evidence of increased peristalsis, that is, loose stools, and at autopsy the gastrointestinal tract was empty and there was increased motility. These animals ate well up to the last few hours of the experiment, but lost 12 to 18% of their original body weight. The animals receiving both alpha cell extract and sugar ate poorly for the first few hours after the sugar injection was started but subsequently ate well and at autopsy the gastrointestinal tract was not empty. These animals showed no loss in weight at the end of the experiment. The gastrointestinal tract of the fasted animals was empty at autopsy. Fasting produced a loss in weight of 9 to 11% in the control animals.

## CYTOLOGICAL STUDIES OF THE LIVER AND PANCREAS

1. After 24 hours of alpha extract (without dextrose) almost every cell in the liver contained some fat and many

cells were loaded with it (fig. 1). The fat was distributed throughout the lobule. Where it occurred as droplets in the cell, the distribution was toward the sinusoid. Tests for glycogen were uniformly negative.

In the islands of Langerhans, from one-third to one-half of the beta cells were small and agranular, in the others the granules occurred at the pole of the cell next to the capillary. The alpha cells were loaded with atypical granules (fig. 13). The acinar cells were vacuolated, which was due in part to swollen and fatty mitochondria.

2. After dextrose, followed by the combination of dextrose and alpha extract, no fat was detected in the liver (fig. 2). Almost every cell contained some glycogen but the cells were not greatly distended by it.

In the islands of Langerhans, about half of the beta cells were poor in granules and the remainder contained darkly-staining granules peripherally arranged (fig. 9). Some mitoses were found in the beta cells. The alpha cells appeared to be reduced in number. The majority of them were loaded with atypical granules, a few were agranular (fig. 14). The zymogen granule content of the acinar cells was reduced. These cells were not vacuolated and the mitochondria were in some cells only slightly swollen.

3. The first biopsy of the liver from this animal, taken before the beginning of the injection after the animal had been fed for 24 hours following a 24-hour fast, showed a moderate content of fat (fig. 3). The second biopsy of the liver, taken after 24 hours of continuous injection of alpha extract, showed an increase in fat content and some fat in almost every cell (fig. 4). The liver removed after 24 hours of combined alpha extract and dextrose, contained less fat than at the beginning of the experiment (fig. 5). At this time almost every cell contained some glycogen although the cells were not distended by it.

In the islands of Langerhans only a few beta cells were poor in granules. The majority contained darkly-staining granules, peripherally arranged. The alpha cells did not appear to be reduced in number and were loaded with their characteristic granules. The acinar cells were moderately vacuolated, their mitochondria moderately swollen and fragmented. The zymogen granule content was somewhat reduced.

4. a. A guinea pig from the older group, sacrificed after a 36-hour fast, showed considerable fat in the liver (fig. 6). The distribution in the lobule was not uniform. The greatest content of fat occurred in the periphery of the lobule, that is, in the region of the portal venules. The cells were uniformly negative for glycogen.

In the islands of Langerhans, the beta cells were uniformly filled with granules distributed throughout the cytoplasm of the cell (fig. 10). The granule content of the alpha cells was variable. A few cells exhibited a normal granule content but these contained small fat droplets. Many were agranular and a few vacuolated. The mitochondria in all the alpha cells appeared normal. The acinar cells were loaded with zymogen granules, their mitochondria fragmented, swollen and fatty.

b. A guinea pig from the younger group, sacrificed after 48 hours fast, contained a moderate amount of fat in the liver (fig. 7). The fat was largely at the periphery of the lobule and here it occurred in the form of large globules. Toward the central vein some cells contained fat in fine droplet form. The liver cells were uniformly negative for glycogen.

The islands of Langerhans and the acinar cells of the pancreas exhibited the same changes as those from the preceding animal.

The data from these experiments are condensed, for convenience and comparison, in the following table:

| EXPERIMENT | LOSS IN WEIGHT | BETA CELLS | ALPHA CELLS | LIVER GLYCOGEN | LIVER FAT |
|---|---|---|---|---|---|
| 1. Alpha extract 2 cc. per hour for 24 hours | 12–18% | Moderately exhausted | Loaded with atypical granules | None | In almost every cell |
| 2. Sugar 24 hours. Sugar plus alpha extract 72 hours | None | Many exhausted; some mitoses | Reduced in number; some loaded with atypical granules; a few agranular | Considerable | None |
| 3. Alpha extract 24 hours. Alpha extract plus sugar 24 hours | None | Small; some exhausted | Loaded with granules | Considerable | 1st biopsy, moderate amount;<br>2nd biopsy, increased;<br>3rd less than 1st |
| 4. Fasted animals | 9–11% | Small well filled with granules | A few normal; many agranular; some vacuolated | None | Large amount in older animals; moderate amount in younger animals |
| Woerner ('38) dextrose, about 1.0 gm. per kilo per hour | 20–30% | Many exhausted but increase in number of beta cells | Exhausted and hydropic | Very little | Almost none |

## MORPHOLOGICAL EVIDENCES OF THE FUNCTIONAL STATE OF THE ALPHA AND BETA CELLS OF THE ISLANDS OF LANGERHANS

Before an attempt to interpret these results can be made, the cytological characteristics of the alpha and beta cells of the islands of Langerhans should be considered. Lane ('07) first showed the histological differences and distribution of these cells in the guinea pig. Bensley ('14–'15) has emphasized the cytological methods by which these cells should be studied. Bloom ('31) (confirmed by Thomas ('37), and others) has demonstrated an additional type of cell in the islands with the azo-carmine staining method. Hunt ('37) has made differential counts of three types of cells in the islands of dog pancreas. Beams ('30) has described the Golgi and canalicular apparatus, vacuome and mitochondria in the island cells of the albino rat. O'Leary ('30) described the living island cells of the mouse at rest and during secretion. Ludford and Cramer ('27) described changes in the Golgi net which they believed were due to the functional state of the island cells. But their criteria for activity are not convincing and their deductions have not been confirmed by subsequent investigators. All of these observations indicate that there is a considerable range of normal variation in the cell size, distribution of granules, mitochondria and Golgi apparatus in these cells. But in general little distinction has been made between the alpha and beta cells. Because of marked differences which were observed in these cytological constituents under the conditions of the experiments reported here, it seemed necessary to determine the normal variation in each type of cell in order to evaluate these changes.

For the study of cell size, granule content and 'negative' Golgi apparatus, that is the system of clear canals, fixation in formalin-chrome-sublimate and 4 μ sections stained with neutral gentian were the most serviceable. Mitochondrial studies were made on 4 μ sections of material fixed in acetic-osmic-bichromate and stained with aniline acid fuchsin and methyl green.

In the islands of the 'normal' control animals there was a considerable range of variation in all the cell constituents of both alpha and beta cells as can be seen in figure 8 and Woerner ('38, pp. 52, 53, figs. 4, 4a). This variation if it represents various stages of the cytomorphosis and functional state of the cells, is to be expected. The alpha cells are for the most part larger than the beta cells, with oval clear nuclei containing a prominent nucleolus and only a few chromatin knots. The alpha granules after formalin-chrome-sublimate fixation and staining with neutral gentian are less discrete than the beta granules and tend to aggregate. With optimum differentiation of neutral gentian they approach a magenta color. The distribution of the granules is quite variable but tends to be rather uniform throughout the cytoplasm. The Golgi net is clearly visible in most of the cells.

The mitochondria are variable in shape but tend to be short fine rods, uniformly distributed through the cytoplasm.

The variation in size of the beta cells may be due in part to the plane of section. The nuclei are smaller, more spherical than those of the alpha cell and contain several chromatin knots. The granules are discrete, vary somewhat in size and distribution, but tend to be uniformly distributed throughout the cytoplasm, except for the Golgi canals. With good differentiation they stain blue with neutral gentian. The Golgi net is clearly visible in most of the cells. The mitochondria are small oval granules, a little larger than the beta granules, uniformly distributed through the cytoplasm.

Considerable deviation from the normal variation of these cytological characteristics after continuous intravenous injection, indicates that the functional state of the cell may be expressed in these structural changes. Let us consider the morphological and physiological evidences for this correlation first for the beta cells and then for the alpha cells.

The first response of the beta cells to continuous intravenous dextrose is the loss of granules without any change in size over the normal variation. The Golgi net is clearly visible and the mitochondria appear within the range of normal variation as to shape and distribution. This condition we have interpreted as primary exhaustion (Woerner, '38, figs. 5, 5 a).

After prolonged injection of up to 2 gm. per kilogram of body weight per hour of dextrose, the initial phase of exhaustion is followed by a compensatory hyperplasia and hypertrophy of the beta cells. The former is evidenced by mitosis in beta cells and the transformation of other pancreatic cells into beta cells. The hypertrophy is evidenced by increase in the size of the beta cells over the normal range of variation. This hypertrophy is accompanied by other cytological changes. The beta granules are slightly larger and stain more intensely. They are distributed at the poles of the cell toward the capillary and are arranged in linear groups parallel to the long axis of the cell. This is associated with the linear arrangement of the slightly larger mitochondria. The Golgi net is clearly visible. This appearance of the cell we have interpreted as evidence of active secretion (Woerner, '38, figs. 6 to 10).

After several days of injection of 2 gm. or more of sugar per kilogram of body weight per hour either alone or combined with alpha cell extract, the beta cells are of two types, one the hypertrophied actively secreting type described above, the other a large argranular cell, vacuolated, with mitochondria larger than normal and clearly visible Golgi net. These cells have a characteristic beta cell nucleus. This type we have interpreted as an exhausted cell with beginning hydropic degeneration. This type of cell has also been described by Homans ('15) and Allen ('22) after subtotal pancreatectomy. These two types of cells are illustrated in figure 9.

After fasting, the beta cells are uniformly small. The size lies within the normal range of variation but in the fasting animal the variation is considerably less. The granules are small, stain poorly and are evenly distributed throughout the cell. The mitochondria are small and distributed uniformly throughout the cytoplasm. The Golgi nets are difficult to find, but in some cells are clearly visible. This condition we have interpreted as an inactive cell (fig. 10).

Changes in the alpha cells are on the same order but occur under different conditions. After several days of injection of about 1 gm. of dextrose per kilogram of body weight per hour the alpha cells are large and agranular, many of them are vacuolated. The mitochondria are thin rods and the Golgi net is clearly visible (fig. 11). This appearance we have interpreted as exhaustion and beginning hydropic degeneration (Woerner, '38, figs. 8, 8 a, 9, 9 a).

After several days of injection of 2 gm. dextrose per kilogram of body weight per hour, the alpha cells are of two types. One type of cell is large and contains granules at the pole of the cell toward the capillary. These granules occur in linear groups, associated with the linear arrangement of the mitochondria which are thin rods. The Golgi net is clearly visible (fig. 12). This condition we have interpreted as an actively secreting cell. The other type is what we have designated the exhausted cell, described above.

After 24 hours of injection of alpha cell extract, the large alpha cells are loaded with granules. The granules are grouped and in some cases appear to have coalesced. In one pancreas exhibiting this type of alpha cell granules, the granules were removed by alcohol fixation, whereas in the normal alpha cell the granules are preserved by alcohol fixation. These granules are distributed throughout the cytoplasm of the cell. The mitochondria are not distinguishable and the Golgi net is not often seen (fig. 13). This condition we have interpreted as suppression of secretion.

In one animal injected for several days with both dextrose and alpha cell extract another type of alpha cell was occasionally found. These cells were somewhat smaller, devoid of granules, with no vacuolation of the cytoplasm, and had a characteristic alpha cell nucleus. The mitochondria were somewhat more rounded and diffusely distributed in the cytoplasm and the Golgi net was rarely visible (fig. 14). This appearance was interpreted as a further suppression of secretion, perhaps beginning atrophy or dedifferentiation.

These observations seem to illustrate the sequence of changes in cytological characters as a result of continued stimulation on the one hand and suppression on the other. In the first case it appears that continued stimulation causes exhaustion followed by increased activity which in turn is followed by hydropic degeneration. With suppression of secretion by substitution therapy (or inhibition) it appears that the secretory antecedents accumulate in the cell, undergo physical changes and are finally lost so that the cell is either dedifferentiated or becomes atrophied. This is essentially the law of suppression formulated by R. R. Bensley (personal communication), based on his studies of experimental changes in the thyroid gland and on adenoma of the eosinophilic cells of the anterior lobe of the hypophysis.

As yet no similar changes due to suppression of secretion have been observed in the beta cells. Theoretically these should occur in normal animals injected with insulin, or in islands of a pancreas containing a recent neoplasm of the beta cells.

## DISCUSSION

Because of our lack of knowledge concerning intermediate fat and carbohydrate metabolism, it is impossible to completely analyze and interpret all the results of these experiments. However, the following interpretations seem to be justified from the results of this series in conjunction with the results after continuous injection of dextrose alone reported in an earlier paper (Woerner, '38) (see table).

The exhaustion and degeneration of the alpha cells, and the excessive loss of body weight resulting from the continuous injection of moderate amounts of sugar alone is prevented when alpha cell extract is injected with the sugar.

The loss of weight and increased deposition of fat in the liver resulting from continuous injection of alpha cell extract alone is prevented when sugar is injected with the alpha extract.

The loading of the alpha cells with atypical granules following injection of alpha cell extract may be interpreted as suppression of the secretion of the alpha cells by the substitution of their secretory products in the blood stream. This may prove to be an index of the potency of the alpha cell extract.

The increase in fat in the liver following injection of alpha extract cannot be due entirely to fasting, for the increase is greater than that in the fasted animal, the distribution more uniform, and these animals were not fasted, except perhaps for the few hours at the end of the experiment when they ate poorly.

The differences in the results obtained in experiments 2 and 3 may be due to several factors. In experiment 2 both the sugar and alpha extract were injected for a longer period of time. The apparent reduction in the number of alpha cells may be evidence of the further effect of the suppression by substitution therapy. In experiment 3, 24 hours of feeding was apparently not sufficient to remove all the fat accumulated in the liver as a result of fasting. The increase of fat in the liver, though significant was not as profound as in some of the older animals. This may have been due in part to intereference in liver function resulting from the biopsy. This is a factor usually ignored in physiological experiments. That it is an important factor is substantiated by the observations of M. H. Knisely in the direct examination of the liver by means of quartz rod illumination. These indicate that such a trauma to the liver may completely upset the normal circulation in this organ (personal communication). The smaller increase in fat may also have been due to difficulties encountered in the preparation of the alpha extract used in this experiment. These factors and the shorter period of administration of dextrose may account for the slight residual fat in the liver at the end of the experiment.

No definite correlation between the fat in the liver in fasted animals and the condition of the alpha cells can be made at present. Such a variable content of alpha granules might represent the intermediate stage of either exhaustion or suppression or a combination of both. However, the normal appearance of the mitochondria in even the agranular alpha cells and the vacuolation of the cytoplasm in these would favor the interpretation of activity rather than suppression. The absence of glycogen in the liver and the appearance of the beta cells would indicate a diminished carbohydrate metabolism.

It has been known for some time that completely depancreatized dogs, maintained with insulin, usually develop progressive fatty infiltration of the liver, which becomes fatal. Dragstedt, Prohaska and Harms ('36) have shown that an extract of the pancreas, administered by mouth, decreased the fat in such liver and protected the depancreatized animals against a fatal fatty infiltration. To the specific substance contained in this extract they have given the name 'lipocaiac,' to designate a fat-metabolizing hormone. They have also shown (Prohaska, Dragstedt and Harms, '36) that this substance is not contained in the pancreatic juice.

It seems probable that the active substance in their extract is essentially the same as that of the alpha cell extract described in this paper. Although most of their experiments were made with an alcoholic extract from which the fats had been removed with ether, it is significant to note that the greatest effects reported by them were produced by the "salt solution extract of pancreas residue." Moreover, in analyzing their procedure, it becomes apparent that in the process of extraction, considerable water, in the form of tissue fluid was added to the 95% alcohol. It may be that some of the alpha granules are removed with more dilute alcohols, either by physical disintegration or solution. We have observed that alpha granules preserved with alcohol may subsequently be removed with water. This has been further tested by extracting the pancreas with alcohol prior to making the aqueous extract. And in this case the results of injection of the aqueous extract were not as profound or striking. Further evidence that the aqueous extract contains more of the active principle than the alcoholic extract is based on the dosage used in the two groups of experiments. The dosage of alpha cell extract computed in terms of grams of fresh pancreas per kilogram of body weight per day used in the experiments reported here, was about half the dosage of alcoholic extract of pancreas, computed in the same way, used by Dragstedt and his co-workers.

If the active principle is the same in both types of extract, it would seem at first glance that the action of the specific substance was directly opposite in the two types of experiments. That is, in the intact animal the fat in the liver is increased after alpha extract injection, whereas in the depancreatized animal the fat in the liver is removed with the administration of lipocaiac. This contradictory evidence can only be explained by the relation of carbohydrate to fat metabolism, which has long been recognized as an intimate one.

It would seem from these experiments that the complete oxidation of fat depends not only on lipocaiac or the product of the alpha cells but also on oxidizable sugar. This seems to be borne out by both types of experiments. In the intact animal the fat stored in the liver after injection of alpha extract is removed when sugar is injected simultaneously and the beta cells appear to be actively secreting. In the depancreatized dog, the sugar is made available for oxidation by the administration of insulin and the product of secretion of the alpha cells is probably contained in the lipocaiac.

What part the fat-metabolizing hormone plays in the oxidation of fat is not clear. Evidently it can play only an indirect role in the mobilization of fat and its deposition in the liver, since this is accomplished in the depancreatized animal. It has been suggested that the hormone is concerned with the desaturation and beta-oxidation of fat in the liver. This suggestion has much to commend it, but more evidence is needed before it can be accepted.

## SUMMARY AND CONCLUSIONS

An aqueous extract of the guinea pig pancreas, freed of beta and zymogen granules by centrifugation and of fat by ether extraction filtered through a Berkefeld filter and injected intravenously into a guinea pig at the rate of 2 cc. per hour for 24 hours produced an increase of fat in the liver, a loss of liver glycogen and a loading of the alpha cells of the islands of Langerhans with granules.

When sugar was injected with the alpha cell extract the fat was removed from the liver, the liver cells stored glycogen, the beta cells appeared actively secreting and somewhat exhausted, but the alpha cells were loaded with granules.

Animals fasted for 36 and 48 hours exhibited considerable fat in the liver, no liver glycogen, the beta cells appeared inactive, and the alpha cells varied from normal to actively-secreting types.

These experiments suggest that: 1) The alpha cells secrete a substance that is concerned with fat metabolism. 2) The injection of an extract of the alpha cells inhibits or suppresses the secretion of the alpha cells. 3) The oxidation of fat depends not only on the presence of such a substance, but also on available oxidizable sugar. 4) An intimate relation exists between the alpha and beta cells, fat and glycogen in the liver, and oxidizable fat and oxidizable sugar.

We wish to express our gratitude to Prof. R. R. Bensley for his interest and advice throughout the work, to Prof. G. W. Bartelmez for his valuable suggestions, and to R. D. Bensley for the photomicrographs.

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# PLATES

## PLATE 1

### EXPLANATION OF FIGURES

Photomicrographs of surface sections of liver fixed in A.O.B. and cut $4\,\mu$ thick. Unstained. × 181.

1 From a guinea pig of the older group (a), weighing over 500 gm., after 24-hour injection of alpha cell extract.

2 From a guinea pig after continuous injection of dextrose followed by combined sugar and alpha cell extract for 3 days.

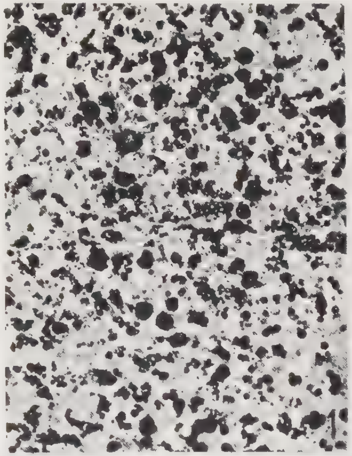
3 First biopsy of liver before injection after 24 hours of feeding following 24-hour fast.

4 Second biopsy of the same liver after 24-hour injection of alpha cell extract.

5 From the same animal after 24 hours of combined alpha cell extract and sugar.

6 From a guinea pig of the older group (a), weighing over 500 gm., fasted for 36 hours. Compare with figure 1.

7 From a guinea pig of the younger group (b), weighing between 300 and 400 gm., fasted for 48 hours. Compare with figure 4.



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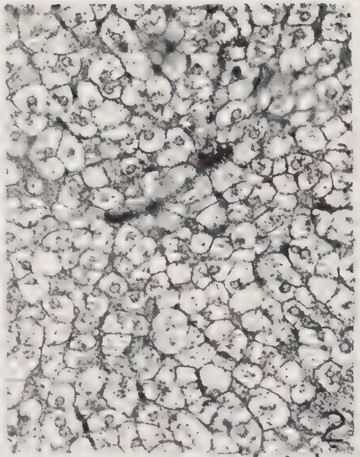

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## PLATE 2

### EXPLANATION OF FIGURES

Camera lucida drawings of alpha and beta cells showing functional variations in cytological characteristics. From sections of pancreases fixed in formalin-chrome-sublimate, cut 4 μ thick, stained with neutral gentian. × 1600.

8 Alpha and beta cells from two 'normal' control pancreases, showing variation in cell size, granule content and distribution, and Gogi net. a, alpha cell; b, beta cell; g.n., Golgi net.

9 Beta cells from the pancreas of an animal receiving 2 gm. of dextrose per kilogram of body weight per hour and 2 cc. of alpha cell extract per hour for 3 days. a.s.b., actively secreting beta cell; e.b., exhausted beta cell; g.n., Golgi net; v., vacuoles.

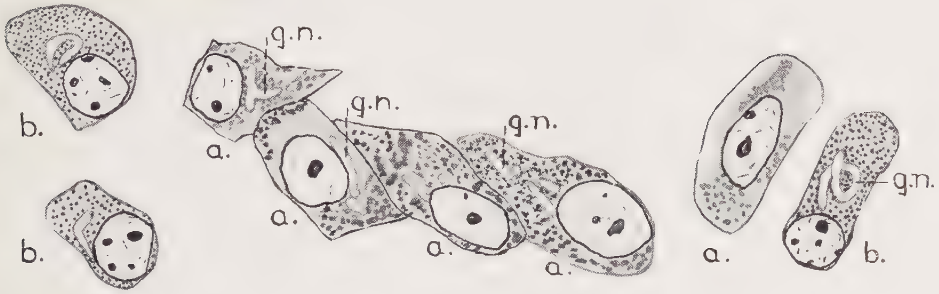
10 Beta cells from the pancreas of a fasted animal showing variations in cell size, granule content, and Golgi net.

11 Alpha cell from the pancreas of an animal receiving 1 gm. of dextrose per kilogram body weight per hour for 7 days. g.n., Golgi net; v., vacuoles.

12 Alpha cell from the pancreas of an animal receiving 2 gm. of dextrose per kilogram body weight per hour. g.n., Golgi net; c.p., capillary pole of cell.

13 Alpha cells from the pancreas of an animal receiving 2 cc. of alpha cell extract per hour for 24 hours.

14 Alpha cells from the pancreas of an animal receiving 2 gm. of dextrose per kilogram body weight per hour plus 2 cc. of alpha cell extract per hour for 3 days.



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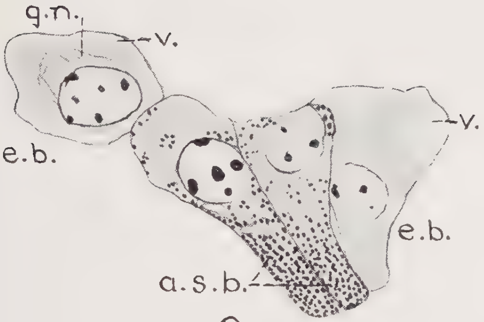

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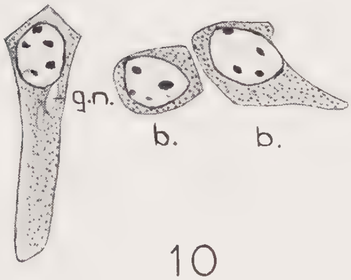

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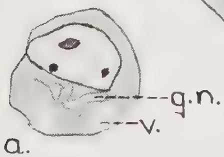

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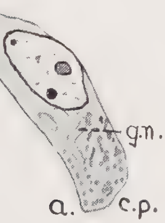

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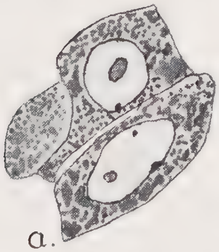

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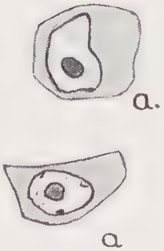

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# PERIODIC AND RHYTHMIC MITOTIC ACTIVITY IN THE KIDNEY OF THE ALBINO RAT

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TWO FIGURES

The morphology of mitosis has been studied extensively. In unicellular and some of the simpler multicellular organisms the factors regulating it have been investigated. Experiments have been conducted in which stimulation and inhibition of this form of cell division have been attempted. Mitogenetic radiation is the object of much attention. Few studies have been made concerning the physiology of this process in higher forms. Mitosis is probably the commoner form of cell division in most tissues and organs. As such, it is the essential feature of growth by hyperplasia, and knowledge of the manner in which it is controlled would be of importance in both normal and abnormal increase.

Great difficulties surround the problem. Although removal of an organ, part of an organ, or a few cells to an artificial medium makes direct observation feasible, it eliminates the influence of the rest of the organism, which is of obvious importance in normal mitotic activity. Direct microscopic observation of parts of the living organisms is reduced in value by the limited areas which can be studied, and by the abnormal conditions which are sometimes created.

## MATERIALS AND METHODS

As an approach to the problem it was decided to determine whether mitotic activity was rhythmic or periodic. Such studies have been made in mammals by Fortuyn-van Leyden

('17, '26), Ortiz Picón ('33), and Carleton ('34). Their observations will be discussed later. For this purpose 240 male albino rats of the Wistar strain, highly inbred, were employed. When 28 days old they were killed with chloroform, the executions having been timed so that twenty rats were killed sometime within each 2-hour interval of the day and night. When litter mates were used they were killed at equidistant points in the 24-hour period so that inherited differences in rate of growth would not by chance be concentrated at certain times. The rats were autopsied as rapidly as possible, and usually all parts were removed and in the fixative within 5 minutes after death. With the exception of the adrenal and thyroid glands, which were fixed whole, thin slices, to insure rapid fixation, were taken of the following structures: brain, liver, kidney, pancreas, skin, small intestine, submandibular gland, testis, thymus and tongue. Bouin's fluid was employed as the fixative. Dehydration, clearing, infiltration with and embedding in paraffin were done in the usual manner.

Thus far the cortex of the kidneys from the first 96 rats has been studied. Each 2-hour interval was represented by eight rats. From the left kidney of each rat a central segment, approximately 5 mm. thick, was sectioned serially at 8 μ. Every twentieth section was mounted. Heidenhain's iron hematoxylin was used first, but trial showed that careful staining with Harris' hematoxylin and eosin Y, much less time-consuming, gave preparations as good for counting mitotic figures. As an index of the mitotic activity of a specimen the number of mitoses in 1000 fields was counted. A field was demarcated by two pairs of hairs arranged to form a square and cemented in a cardboard ring set in the eyepiece of a microscope. The counting was done at a magnification of 1000 diameters. To make the findings more representative of a kidney, instead of counting 1000 consecutive fields in a single section, 250 consecutive fields in each of four sections were examined. The four sections studied were usually the first, fifth, tenth and fifteenth, and since each of these sections was every twentieth of the serial ribbon, there was an interval of at least 480 μ between them. Thus there was no danger of counting the same mitosis twice. Counting was always begun at the lateral border of the kidney so that approximately the same portion of each kidney was inspected. The findings were recorded as number of mitoses per 1000 fields. In this way values of mitoses per 1000 fields of renal cortex were obtained from each of eight rats for each 2-hour interval. The values for individual rats are listed in table 1 according to the time the rats were killed. The means of the values for each 2-hour interval were calculated (table 1) and from these a curve was constructed. The points on the curve representing mean values were placed midway in the corresponding time intervals; i.e., the average value for the period 8 P.M. to 10 P.M. was placed at 9 P.M. The curve is presented in figure 1.

TABLE 1

*Individual and mean values of mitoses per 1000 fields, based on eight rats per 2-hour interval for a period of 24 hours*

| 12–2 P.M. | 2–4 P.M. | 4–6 P.M. | 6–8 P.M. | 8–10 P.M. | 10–12 P.M. | 12–2 A.M. | 2–4 A.M. | 4–6 A.M. | 6–8 A.M. | 8–10 A.M. | 10–12 A.M. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 36 | 66 | 38 | 58 | 61 | 12 | 40 | 30 | 30 | 60 | 24 | 33 |
| 63 | 47 | 42 | 45 | 31 | 18 | 25 | 35 | 41 | 28 | 63 | 25 |
| 44 | 41 | 71 | 51 | 22 | 54 | 32 | 42 | 27 | 35 | 64 | 40 |
| 72 | 35 | 25 | 73 | 59 | 21 | 59 | 83 | 40 | 41 | 28 | 102 |
| 62 | 36 | 44 | 26 | 27 | 21 | 65 | 63 | 42 | 41 | 39 | 42 |
| 17 | 68 | 59 | 74 | 12 | 22 | 47 | 53 | 54 | 39 | 53 | 33 |
| 47 | 67 | 54 | 40 | 37 | 46 | 27 | 27 | 47 | 58 | 58 | 25 |
| 103 | 122 | 35 | 36 | 20 | 34 | 33 | 13 | 23 | 55 | 57 | 31 |
| 55.5 | 59.8 | 46.0 | 50.4 | 33.6 | 28.5 | 41.0 | 43.3 | 38.0 | 44.6 | 48.3 | 41.4 |

## OBSERVATIONS

The data show that in the cortex of the kidneys studied there was periodicity of mitotic activity. The lowest rate of activity occurred during the period from 10 P.M. to 12 night, the mean value being 28.5 mitoses per 1000 fields. The highest rate of activity occurred during the period from 2 P.M. to 4 P.M., the mean value being 59.8 mitoses per 1000 fields. From the point of lowest to the point of highest activity there was an interval of 16 hours; from high to low point, 8 hours.

The irregular curve seems to be composed of 6-hour cycles. Starting from the lowest point of mitotic activity at 11 P.M., there is a rise to 1 A.M., another rise to 3 A.M., then a drop to 5 A.M. In the next 6-hour interval there is a rise in mitotic activity from 5 A.M. to 7 A.M., another rise from 7 A.M. to 9 A.M., then a drop to 11 A.M. In the third 6-hour interval there is a rise from 11 A.M. to 1 P.M., another rise to 3 P.M., then a drop to 5 P.M. But in the last 6-hour interval there was a rise from 5 P.M. to 7 P.M., then a fall to 9 P.M., and a second fall to 11 P.M., the lowest point of activity during the 24-hour period. The similarity in character of curves representing three of the 6-hour intervals, and the striking departure from these of the fourth is graphically portrayed in figure 2.

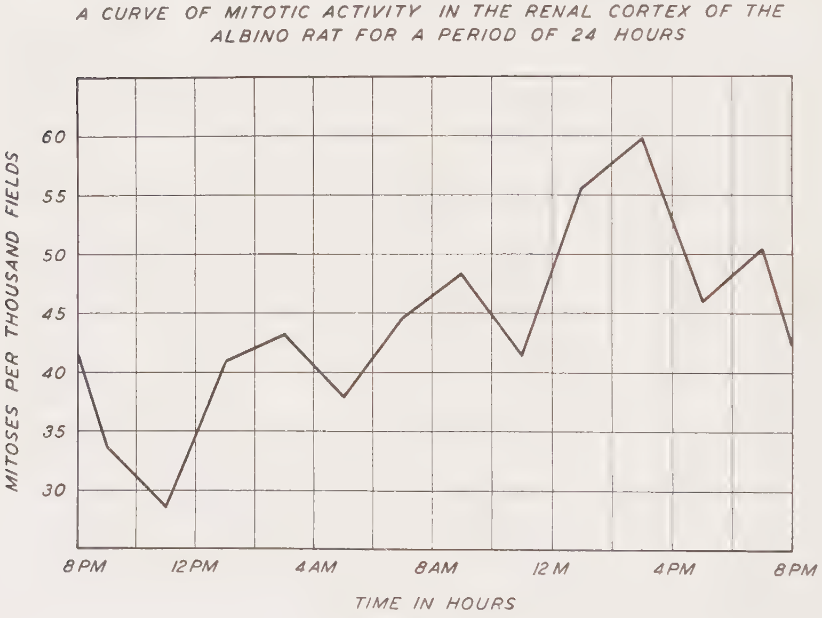

Figure 1

Table 1, in which individual values are listed, shows that for any period there is wide variation of these values. Presentation of means, alone, gives too little consideration to the variability. In making a statistical study of the data, using standard biometric methods, comparisons were drawn between mitotic activity during the day and night, and between the first and second halves of each of these intervals. The period from 8 A.M. to 8 P.M. was designated as day; that from 8 P.M. to 8 A.M., as night. These periods, divided in half, were designated, respectively, as: first half of day, second half of day, first half of night and second half of night. Mean values and statistical data for comparisons made between various periods are given in table 2.

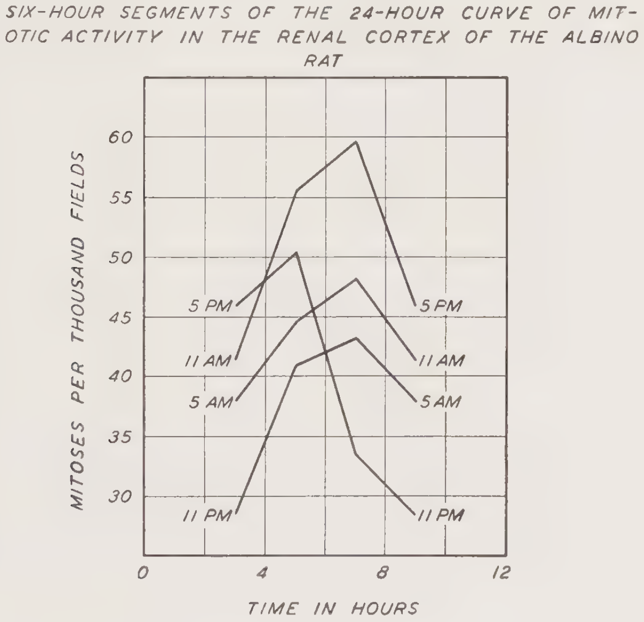

Figure 2

From these data it was learned that the mean level of mitotic activity was significantly greater during the day than at night. Mitotic activity was at its lowest level during the first half of the night, and rose, by gradually decreasing steps, to the highest level during the second half of the day. Comparisons drawn between successive halves of the night and day revealed no significant differences. Mitotic activity was significantly lower during the first half of the night and significantly higher during the second half of the day than during the rest of the 24-hour period.

TABLE 2

*A statistical comparison of average levels of mitotic activity during various portions of the 24-hour period*

| TIME INTERVALS COMPARED | | | | | | DIFF. ±P.E. | S.R. |
|---|---|---|---|---|---|---|---|
| Time | No. | Mean±P.E. | Time | No. | Mean±P.E. | | |
| | | | *vs.* | | | | |
| 8 P.M. to 8 A.M. | 48 | 38.16±1.54 | 8 A.M. to 8 P.M. | 48 | 50.20±2.08 | —12.04<br>±2.58 | 4.6 |
| 8 P.M. to 2 A.M. | 24 | 34.37±2.16 | 2 A.M. to 8 A.M. | 24 | 41.95±2.06 | —7.58<br>±2.99 | 2.5 |
| 2 A.M. to 8 A.M. | 24 | 41.95±2.06 | 8 A.M. to 2 P.M. | 24 | 48.37±3.03 | —6.42<br>±3.67 | 1.7 |
| 8 A.M. to 2 P.M. | 24 | 48.37±3.03 | 2 P.M. to 8 P.M. | 24 | 52.04±2.79 | —3.67<br>±4.12 | 0.9 |
| 8 P.M. to 2 A.M. | 24 | 34.37±2.16 | 2 A.M. to 8 P.M. | 72 | 47.45±1.57 | —13.08<br>±2.67 | 4.9 |
| 2 P.M. to 8 P.M. | 24 | 52.04±2.79 | 8 P.M. to 2 P.M. | 72 | 41.56±1.49 | 10.48<br>±3.16 | 3.3 |

No., number of renal cortices examined. Mean, mean number of mitoses per 1000 fields. P.E., probable error. Diff., difference. S.R., significance ratio, an expression of the probability that an observed difference is due to random sampling. The greater the significance ratio the less likely it is that the observed difference is accidental. In this report a significance ratio of 3 or more is assumed to indicate significance because there is less than 1 chance in 20 that such a difference could occur by chance.

## DISCUSSION

It has been established that mitotic activity exhibits periodicity in rate in various plants. From such studies Fortuyn-van Leyden ('17), was led to investigate mitotic activity in animal tissues. She used six kittens, killed on the second day after birth, and counted the number of mitoses seen in sections of the cornea, small intestine, liver, lymph node, thymus, spleen and bone marrow, and in spreads of the mesentery. Her method of obtaining an index of mitotic activity was to count from 2500 to 10,000 nuclei, and note the number that were in mitosis. Mitoses were so few and difficult to recognize in the liver that she omitted it from further consideration. In the cornea, activity was greatest at 10.30 P.M., least at 10.30 A.M.; in the crypts of Lieberkuhn of the small intestine, greatest at 10.30 P.M. and least at 10.30 A.M.; in the mesentery, greatest at 2.30 A.M. and least at 10.30 A.M. The findings in the lymph node, thymus, spleen and bone marrow were essentially similar. The same investigator reported ('26) studies of mitotic activity in the crypts of Lieberkuhn and cornea of mice 2 weeks old. No mitoses were seen in the cornea, but in the crypts of Lieberkuhn mitotic activity was greatest at 10.30 A.M. and least at 2.30 A.M. Her results are open to the criticism of paucity of samples. Ortiz Picón ('33) in the course of an investigation of mitotic activity in the epidermis of mice of various ages, made some observations concerning daily variations in rate of activity. He utilized skin from the lumbar region of six mice, grouped in three pairs. One mouse from each pair was killed at 12 M., the others at 7 P.M., 8 P.M. and 12 night. An index of mitotic activity was obtained by counting all the mitoses in 1 sq.mm. of skin. Mitoses in the epidermis were more than three times as numerous in the mice killed at noon than in those killed at night. His results, also, are subject to the criticism of too few samples. Carleton ('34) reported studies of mitotic activity in the epidermis and epithelial cells of the hair follicles of forty-eight mice killed from 8 hours to 7 days after birth. She employed eight litters of six mice each, and killed the members of each litter at 4-hour intervals, beginning at 12 M. Five thousand cells were counted in each specimen. The per cent of them in mitosis was considered an index of mitotic activity. The results indicated that mitotic activity was greatest at 8 P.M. and least at 12 M.

The differences in the results obtained by these investigators may well be due to the small size of the samples used by two of them, to differences in organs examined, and to differences in the ages and orders of mammals employed (cat, mouse). In cats, Fortuyn-van Leyden observed the greatest mitotic activity at night, the least during the day; in mice she found activity lowest at night and greatest during the day. Ortiz Picón found mitotic activity in the epidermis of mice greatest at noon and lowest at night, but Carleton, who used a far larger number of animals, found the opposite situation in the same organ of the same animal. My observations, which showed greatest activity during the day and least at night, agree with the findings of Fortuyn-van Leyden and Ortiz Picón for the mouse. However, comparison of results obtained by various investigators will be of value only when such factors as size of samples, animals used, physiologic age of animals, organs studied, and external conditions have been made more uniform. In one respect the previous and present findings were alike: daily periodicity of mitotic activity was found in various organs of cats, mice and rats.

In the present investigation it is not possible to explain the findings from data thus far available. The sudden drop in mitotic activity which occurred during the transition from day to night is suggestive of a causal relationship, but besides being a matter of the possible direct effects of light, changes are produced in the environment and activities of the rats in an experimental colony. The 6-hour cycles pointed out are indicative of rhythmic mitotic activity, but these, too, cannot as yet be explained. It is felt that the chief value of such studies at the present lies in furnishing a tool for working on the problem of growth by hyperplasia under normal circumstances. If it be established that the mean level of mitotic activity in the renal cortex of the rat is twice as great at 3 P.M. as it is at 11 P.M., then a consideration of every measurable difference between kidneys obtained at these two times may help unravel the problem of the regulation of growth by mitosis.

## CONCLUSIONS

1. The renal cortex of ninety-six male albino rats, 28 days old, was examined for mitotic activity, an index of which was established by counting the mitoses seen in 1000 fields. Eight specimens were obtained during each 2-hour interval of a 24-hour period.

2. Mitotic activity was greatest from 2 P.M. to 4 P.M., and least from 10 P.M. to 12 night.

3. The curve plotted from the mean values appeared to be composed of four 6-hour segments, three of which closely resembled each other in character. This was interpreted as indicating rhythmic waves of mitotic activity.

4. Biometric studies revealed that the mean level of mitotic activity was significantly greater during the day (8 A.M. to 8 P.M.) than during the night (8 P.M. to 8 A.M.); that it was significantly less during the first half of the night (8 P.M. to 2 A.M.) and significantly greater during the second half of the day (2 P.M. to 8 P.M.) than during the rest of the 24-hour period.

5. Application of these findings may prove useful in further studies of the factors regulating growth by mitosis.

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# THE EXTRAHEPATIC BILIARY TRACT OF THE HYRAX

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ONE FIGURE

In connection with a perusal of the literature on the comparative anatomy of the extrahepatic biliary tract of mammals, interesting references to the hyrax were observed. Mentzer ('29 a) wrote that "the hyrax is said by Owen to possess normally a single gall-bladder with two accessory pouches," and again ('29 b) that "the hyrax, as reported by Owen, Macalister, and others, possesses a single gall-bladder with two lateral pouches and a single cystic duct." Recently in a paper by Gorham and Ivy ('38) the statement is made that "thus in the dissections of hyrax, three observers found a gall-bladder; five did not."

In the routine examination of a series of animals I had recorded that there was no gall bladder in hyrax. The references which I had read in clinical literature and which appeared to be at variance with the findings in the specimen dissected, led me to make a survey of the available literature and a detailed study of the extrahepatic biliary tract of hyrax.

I wish to express appreciation for the helpful suggestions of Dr. Thesle T. Job.

## SURVEY OF THE LITERATURE

Pallas (1767, 1778) described the viscera of hyrax from a study of three specimens and said there was no gall bladder. Cuvier (1805) reported similarly, but Owen (1832) commented that "Cuvier had not, at the time of the preparation of that work for the press, himself dissected the hyrax.'' Meckel (1829), Kaulla (1830) and Owen (1832) recorded that there was no gall bladder. Owen had not evidently read of Kaulla's work for he said that since the time of Pallas there had been no other account of the soft parts of hyrax until he reported one specimen in 1832. He commented that there were dilated hepatic ducts, and that ''the united capacities of these receptacles equal an ordinary-sized gall-bladder.'' In these ducts he found small concretions of a bright yellow color. Macalister (1867) said that ''the hyrax, or daman, according to Owen, has one central and two lateral sacs.'' From a study of the original accounts it was seen that neither Owen (1832) nor Macalister (1867) mentioned the presence of a gall bladder in hyrax.

Martin (1835), Brandt (1869), Flower (1872), George (1874), and Rachford (1895) reported that there was no gall bladder. Mentzer ('29) made a table which showed the presence or absence of the gall bladder in twenty-six animals. In the series of papers consulted he was the only one who listed the hyrax as an animal which possessed a gall bladder. His comments, however, were based on descriptions made by Pope on two specimens obtained on an African hunting expedition. In one of these specimens the bile ducts were filled with liver flukes. It was not stated on which specimen the reported measurements were made. Mentzer concluded his description of the biliary tract of hyrax with the statement that ''the description of the gall-bladder in Dr. Pope's two specimens is not clear, however, nor is it possible to interpret his drawing with certainty.''

## RESULTS OF DISSECTION

An examination of the specimen (fig. 1) obtained through the courtesy of the department of zoology of the Field Museum of Natural History showed that there was no gall bladder. There were two hepatic ducts. The right hepatic duct was 19 mm. in length and 6 mm. in diameter. The left hepatic duct was 7 mm. in length and 2 mm. in diameter. The common hepatic duct was 31 mm. in length and had a diameter of 4 mm.

Five interlobular ducts opened into the larger right hepatic duct. Three of these five tributaries opened on the right side of the hepatic duct and two on the left side of the duct. Two had diameters of 2 mm., two of 1.5 mm., and one of 1 mm. The smaller left hepatic duct had only two small interlobular ducts with diameters of 2 mm. each.

The larger dilatation on the right side contained several yellow concretions. In the common hepatic duct there were four small, irregularly shaped concretions. In no place was the common hepatic duct completely occluded by these calcareous masses.

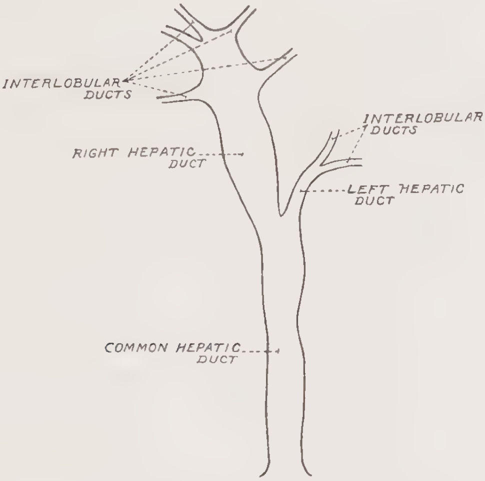

Fig. 1 The extrahepatic biliary tract of the hyrax.

There was no duodenal papilla. The bile and pancreatic ducts opened into a shallow depression which was 22 mm. caudad to the pylorus. The margins of this depression were definitely elevated. It was demonstrated with bristles that the bile and pancreatic ducts opened separately. The pancreas was small and had a second duct which opened 42 mm. caudad to the pylorus.

## DISCUSSION

The dilatation described by Mentzer was undoubtedly just an enlargement of the hepatic duct and not a gall bladder. The drawing of the dilated duct in connection with his report resembles very closely the structure observed in the specimen here described.

The question as to whether there is compensation for the lack of a gall bladder is interesting. Mann, Brimhall and Foster ('20) felt as a result of their studies that the dimensions of the common duct were not related to the presence or absence of the gall bladder. Higgins ('26) called attention to the plexus of bile ducts around the portal veins in the rat's liver, and he suggested that this plexus might be a compensatory mechanism within the liver "for the concentration of bile or for other functions analogous to those of the gall-bladder of the mouse." Schmidt and Ivy ('37) from a study of fourteen species with a gall bladder and four species without a gall bladder were not able to support the hypothesis "that animals possessing a gall-bladder may compensate for its absence by secreting a concentrated bile." The dilatations described by Owen and by Mentzer in the hyrax, and observed in the specimen reported here lead one at first to wonder if these enlargements might be compensatory for the lack of a gall bladder.

It should be noted, however, that in Owen's specimen and in the one here described, calcareous masses were present in the dilatations of the ducts. In one of Pope's specimens described by Mentzer, the bile ducts were filled with liver flukes. Since it was only in these three instances in which dilatations were definitely mentioned, and since these were all associated with pathological conditions in the ducts, it might very likely have been that these enlargements were the result of the presence of calcareous material or of liver flukes in the ducts.

## SUMMARY

1. In a review of a series of reports which included descriptions of the extrahepatic biliary tract of hyrax, it was found that eleven authors reported no gall bladder. Contrary

to references in the clinical literature, neither Owen nor Macalister reported the presence of a gall bladder. In the available accounts of dissections of hyrax no one reported the presence of a gall bladder except Mentzer who based his accounts on descriptions and drawings made by Pope.

2. There was no gall bladder in the specimen of hyrax reported here, but the right hepatic duct was enlarged and contained yellow concretions.

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# STUDIES ON SEX REVERSAL IN AMBLYSTOMA

## XI. REVERSAL IN A. TIGRINUM FEMALES INDUCED BY ECTOPIC IMPLANTATION OF TESTIS PREPRIMORDIA

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TWO TEXT FIGURES AND ONE PLATE (FIVE FIGURES)

### INTRODUCTION

In earlier papers of this series the writer ('29, '31 a, b, c) described a technique (orthotopic implantation) by which one gonadal preprimordium in Amblystoma might be replaced by that of another embryo, and an ovary thus brought under the influence of a testis throughout its entire development, with its consequent inhibition and frequent reversal to a testis. Cases were described in which the supposed reversal was of such complete character that the former ovary had become a testis of adult size and structure ('31 a, fig. 37).

The method of orthotopic transplantation, however, has been criticized by Witschi ('32, '33) who believes that it is likely to result in "mixture of all essential components of host and implant gonads . . . . on both the operated and the unoperated sides." This intermixture, if it actually occurred, would of course result in the formation of mosaics which might readily be interpreted as gonads in reversal.

Recognizing the possibility that intermixture of gonadal components might occur on the operated side through failure to completely eliminate the gonad-forming elements of the host when preparing the graft site, the writer ('29) advocated and practiced a very extensive removal of host mesoderm to preclude such a possibility. Considering the precautions taken, it is doubted that any appreciable number of gonad mosaics could have resulted from host material left in situ. Yet reversal of the gonad developed from the graft, when it happened to be an ovary in a male host, occurred with about the same frequency as reversal of the ovary in females with testis grafts. In such females, obviously, the ovary could become a mosaic of host and implant tissues only through the passage of the latter across the midline at that time in development (preceding and during the formation of the mesentery) when the primordial germ cells and associated elements have been carried farthest medially. Should any transfer of these gonadal components occur, it seems probable that they would cross as frequently in one direction as the other; this would give an intermixture of host and implant tissues on both sides alike. This, theoretically, would explain the similarity in character and fate of graft and host ovaries if one wished to deny the occurrence of an actual reversal in these gonads.

To assert that a translocation of gonad-forming materials never occurs in normal or grafted animals is obviously impossible. Morphological studies, however, indicate that in the urodeles the germ cells of the two sides are not brought together medially and intermingled, but remain on the side of their origin. The writer has undertaken several lines of investigation to test the possibility of their translocation, but has not yet secured satisfactory evidence of its occurrence. In the meantime it is pertinent to inquire whether the supposed reversal of sex following orthotopic implantation can be duplicated by other experimental methods not open to the same objection. One such method, parabiosis, has already been used by the writer ('36), and has served to demonstrate that an actual reversal of ovaries inhibited by testicular influence may occur with considerable frequency. Another method, that of ectopic implantation of gonadic preprimordia, has been recently developed, and is to be described together with certain results secured by it, in the pages that follow.

## MATERIAL AND TECHNIQUE

In the writer's earlier attempts at heterotopic or ectopic implantation of gonadic preprimordia ('28 a, '28 b) the site chosen was one somewhat ventral to the normal position. The resulting ectopic gonads were frequently abnormal in structure, and were usually so small as to be unable to seriously affect the differentiation of the gonads of the host. It seemed useless, therefore, to resort to transplantation into this region as a substitute for orthotopic implantation. The site finally determined to be most suitable for ectopic implantation is one which may be termed pre-orthotopic, i.e., a site rostral to that occupied by the gonad-forming material of the recipient, which in Amblystoma does not usually extend farther cephalad than the level of the eighth or ninth somite. In practice, this site is now limited to the territory of four somites, beginning with that somite whose cephalic margin lies at the cranial border of the pronephric swelling (fig. 1). Since this is preceded by three somites recognizable in sections (Adelmann and Maclean, '34, et al.) it may be termed the fourth somite; the graft site thus corresponds to the territory of somites 4 to 7, inclusive. In preparation for grafting a longitudinal incision is made a short distance above the ventral borders of these four somites. From the cranial end of this cut, an incision is extended ventrally just in front of the pronephric swelling, with a parallel incision at the caudal border of somite 7. The flap of ectoderm and mesoderm now reflected ventrally

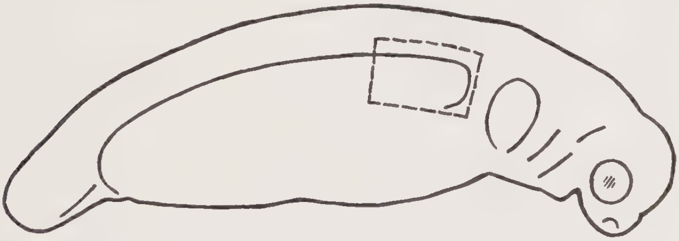

Fig. 1 Diagram of Amblystoma embryo at operating stage to show the implantation site.

and removed includes the material for both the pronephros and the forelimb, which in consequence are absent on the grafted side. The gonad preprimordium to be implanted extends through the territory of somites 8 or 9 to 16, approximately, but the smaller cephalocaudal extent of the somites in this region, and the contraction of the graft as soon as it is removed from the donor, permit it to be readily fitted into the site prepared for it. The operation is performed at stages 30 to 33.

Although this type of ectopic transplantation has now been used in various strains of A. tigrinum, and A. punctatum and the Mexican axolotl as well, the results to be described in this report are only those obtained with A. tigrinum of the strain found near Chicago, this having been the species and strain used most extensively in the earlier work with orthotopic transplants. The relative incidence of reversal in various other strains of A. tigrinum and in other species of Amblystoma is now under investigation and will be discussed in a later report.

An important feature of this study has been the frequency of exploratory operation to determine the condition of the gonads. In the larval animals exploration was carried out under chloretone anesthesia; after metamorphosis, urethane was employed. Of the latter, intraperitoneal injections of 0.5 cc. or more of a 10% aqueous solution were used, the dose depending upon the size of the animal. All autopsy material was fixed in Bouin's fluid; much of it was sectioned serially for microscopic study.

## RESULTS

The gonads developed from ectopic transplants into the preorthotopic region are commonly found immediately rostral to the homolateral gonad of the host. The presence of a gonad preprimordium in this territory in the embryo results in the formation of a genital ridge or fold quite comparable in position, except for its more cephalic location, to the genital fold of the recipient, but usually somewhat shorter in length and of greater diameter in transection. In very young larvae the two genital folds (graft and homolateral fold of host) have always been found discontinuous. Later, however, a narrow ridge usually develops in the interval between them, but the absence or rarity of germ cells in this ridge results in its development into either a thin sterile membrane or a slender constricted region intervening between the two gonads. This often becomes relatively short as the gonads increase in size. Usually, however, a fusion of graft and host gonads so complete as to obscure the boundary between them does not occur except occasionally in females carrying ovarian grafts.

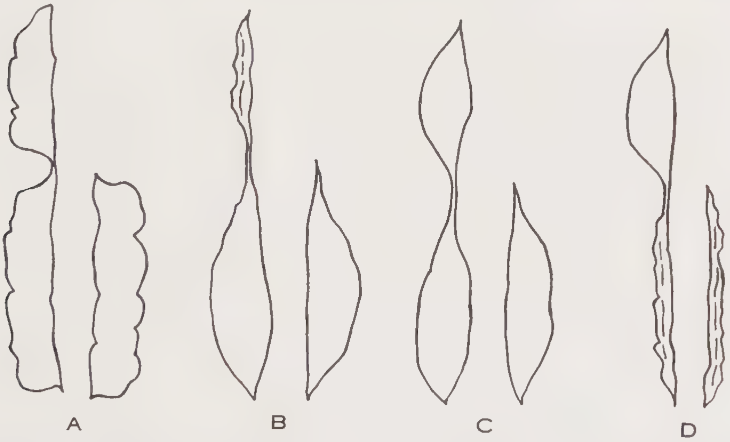

Fig. 2 Diagrams to illustrate the form and relations of the gonads in grafted animals. A, graft ovary in female. B, graft ovary in male. C, graft testis in male. D, graft testis in female.

The usual relations of graft and host gonads are represented diagrammatically in figure 2.

In those males in which the graft is an ovary, its unfavorable ectopic position and its inhibition by the gonads of the host result in its early reduction to a slender atrophic structure, generally much shorter than an ovary normally situated (fig. 2 B). The development of testicular areas in such graft ovaries was not followed in animals of the present series because their occurrence here might be attributed to intermixture of adjacent host tissues with those of the implant rather than to an actual reversal. In females in which the graft gives rise to a testis, the latter, though handicapped by its ectopic situation, nevertheless dominates and inhibits the ovaries of the host in the usual fashion (fig. 2 D). As the right (homolateral) ovary atrophies and the graft testis grows, the latter sometimes expands caudally, invading the cephalic end of the ovary, or fusing with testicular lobes which have developed in that structure. As a result, a right ovary observed at earlier exploration as being comparable in extent to the left, has subsequently been found greatly shortened or, in one instance, completely eliminated. It is advisable, therefore, at the first exploration, when graft and host gonads are found to be united, to remove the region of junction in order that the nature of later changes in the homolateral ovary may not be obscured by its continuity with the graft.

In the animals of the present experiment, the first exploratory operation was performed at ages ranging from 70 to 90 days after grafting, the incision as a rule being made on the right or grafted side. It was found that the four possible combinations with respect to sex were distributed as follows (the small symbol in each case indicating the sex type of the graft): 11 ♂♂ : 15 ♂♀ : 14 ♀♂ : 12 ♀♀. This is a very close approach to the ratio expected when the two sexes occur in equal numbers. Since for the study of reversal in the ovary the animals of most value are females bearing testis grafts, these were retained and animals of the other three groups discarded.

At the time of the first exploration mentioned above, none of the fourteen females carrying testis grafts showed evidence of reversal in the one ovary seen. This ovary in each instance was a very slender, irregular structure, easily distinguishable from a testis or from a normal ovary, even when examined at a magnification of only × 10. It was always examined under higher magnification (× 30) to make sure no early testicular lobules were present in any region.

After an interval of about 2 months the animals, now about 5 months old, were again opened for exploration, the incision this time being made on the left side, and no attempt being made to examine the right ovary. Only one female yet showed evidence of the development of testicular lobules (seminal tubules) in the atrophic left ovary. The animals were at this time somewhat retarded in development for their age, due in part to the previous exploration, else it is probable that reversal would have been found to have occurred in others.

At the third exploratory operation, performed when the animals were 9 months old, four of the surviving thirteen showed distinct testicular areas on the left side with lobules distinguishable when studied at low magnification. Two other animals later developed lobules on this side, bringing the total so doing to 43% of the original fourteen animals.

At subsequent exploratory operations in which the right side was exposed, or at the final autopsy of the animals, it was found that a development of testicular lobules in the right ovary had occurred in eight of the fourteen animals (57%). The occurrence of a more or less extensive reversal, as evidenced by the presence of testicular lobules, may therefore be summarized as follows:

| | |
|---|---|
| Both ovaries | 5 animals |
| Left ovary alone | 1 animal (see protocol of no. 414) |
| Right ovary alone | 3 animals |
| Total | 9 animals (64%) |

The approximate ages at which testicular lobules probably made their appearance in the different animals are shown in table 1; these figures are based in part upon the data secured by exploratory operations and in part upon autopsy findings and comparison with gonads in which the date of reversal was more accurately known. As shown in the table, eight of the nine animals exhibited reversal in one or both ovaries before the end of their first year.

The five animals found not to have developed testicular lobules in either ovary (not listed in table 1) were autopsied at ages of 8, 9, 12, 14 and 18 months. In all of these the ovaries showed germ cells in a hilar or medullary position, associated with efferent ductules or with the longitudinal hilar duct and its branches, which correspond to the rete cord system of the testis. In one ovary of the 14-month animal such germ cells were very numerous (sometimes 10 to 12 per transection). That ovaries such as in these five animals are capable of differentiating further as testes is suggested by the protocol of animal no. 414, below. In addition to that of this animal, the protocols of several other illustrative cases are outlined.

*No. 414.* Ectopic implantation of gonadic preprimordium 3/30/36, at stage 31. Explorations at 5 and 9 months showed the left ovary very slender, without any indication of reversal; from its appearance at 9 months it was judged to be approaching a sterile condition. Exploration of the right side at 11 months showed a long bilobed testis, with the ovary of that side completely lacking. While the caudal lobe of this testis probably originated by reversal in the long atrophic right ovary seen at 2.5 months, this has not been counted as a case of reversal (see table 1). The entire testis was removed. At autopsy 3 months later (age 14 months) the left ovary showed a much thickened hilar region, containing numerous germ cells, with a few lobules in very early developmental stages. The cortex is entirely sterile.

TABLE 1

*Estimated ages at which testicular lobules developed in atrophic ovaries of grafted animals*

| SERIAL NO. | AGE AT AUTOPSY | PROBABLE AGE AT DEVELOPMENT OF LOBULES | |
|---|---|---|---|
| | | Left ovary | Right ovary |
| 402 | 19 months | 4 to 5 months | 12 to 14 months |
| 406 | 16 months | 7 to 8 months | 11 to 14 months |
| 414 | 14 months | 13 to 14 months | ? |
| 431 | 16 months | 7 to 8 months | 7 to 8 months |
| 433 | 9 months | ............ | 8 to 8.5 months |
| 441 | 11 months | 8 to 9 months | 7 to 8 months |
| 455 | 16 months | 10 to 11 months | 14 to 15 months |
| 490 | 17.5 months | ............ | 10 to 12 months |
| 495 | 13 months | ............ | 9.5 to 10 months |

*No. 441.* Ectopic implantation of gonadic preprimordium 4/5/36, at stage 31. Exploration of right side 70 days later showed a graft testis well cephalad of the slender atrophic ovary. Exploration on left at 5 months was negative, but that at 9 months showed a series of nodules in the hilar region of the ovary, made up apparently of very early lobules.

Killed at 11 months because of a rectal obstruction. Both ovaries are well advanced in reversal, the right having one large rounded testicular lobe while the left shows a series of four smaller ones (fig. 3) with early lobules. The graft testis, as found at exploration, is far cephalad in position, with only a narrow, sterile fold between it and the testis derived from the right ovary.

*No. 406.* Early history similar to that of no. 441, except that the area of reversal found in the left ovary at 9 months was limited to its extreme caudal end. At 11 months the very large ectopic testis was removed. The animal was killed at the age of 16 months. In the interval since the exploration at 9 months the entire left ovary had become converted into a series of small testicular lobes (fig. 4); a small lobe had also developed in the caudal part of the right ovary.

*No. 431.* Early history as in no. 441. At the third exploration (at 9 months) the left ovary showed a small testis lobe toward its cephalic end. About 2 months later, when the graft testis was removed, the right ovary was found to have begun reversal also, a testis lobe about 4 mm. long being present. The graft testis was located at a considerable distance from this small testis lobe, and was not connected to it. Five months later, at the death of the animal, its gonads were found to be large testes (fig. 5), differing but little from those of a normal male except that they are rather shorter and broader and the left is bilobed.

*No. 402.* Early history as in no. 441. At 5 months, exploration revealed a possible mass of testicular lobules near the middle of the left ovary; this was confirmed by exploration at 9 months. The ectopic graft testis, about the size of a large kidney bean, was removeed at 11 months. The animal was killed 8 months later (November, 1937) at which time its cloaca was enlarged and highly vascular as in sexually active adult male. The gonads are shown in figure 6. The largest of the four lobes on the left side is probably the one observed at 5 and 9 months, and also seen through the mesentery as an oval mass 2 to 3 mm. in diameter at the time the graft testis was removed The smaller lobes on the left and the irregular testis on the right were not present at that time, but developed during the 8 months which elapsed before autopsy. On sectioning, their lobules are found to contain spermatozoa. The vasa deferentia are large and convoluted (fig. 6). The cloaca, in sections, is that of a normal adult male, with the various glands in active secretory stages.

*No. 455.* Early history similar to that of no. 441 or 406, except that no testicular lobules were found in the left ovary until the exploration 11 months after grafting, when they appeared as a small mass in its middle third. The animal was kept, without removing the graft testis, until the age of 16 months. At autopsy the testicular lobe seen 5 months earlier was found still to be of very small size (fig. 7). On the right a testis lobe had developed in the cephalic end of the ovary and had become partly fused to the graft testis, while a much smaller group of lobules had appeared at the caudal end of the ovary.

*No. 495.* Ectopic implantation of gonadic preprimordium 4/17/36 at stage 31. At the first exploration 3 months later, the long slender atrophic ovary on the right showed no reversal. The left ovary at 5 and 9 months likewise showed no definite testicular structure. The fourth exploration, at 10.3 months, was made from the right side. The ovary was found to have developed a small mass of testicular lobules near its caudal end, and the graft testis was accordingly removed. Almost 3 months later (age 13 months) the animal was again opened on the left side. When it was found that the ovarian cortex on that side had undergone considerable regeneration, the animal was at once killed and autopsied. The testicular mass previously observed in the right ovary was still present, but on microscopic study was found to show degenerative changes. Due to its small size at removal of the graft testis, it had been unable to inhibit cortical development in the atrophied ovaries. It would probably have undergone further degenerative changes as ovarian regeneration continued, had the animal not been sacrificed for study.

## DISCUSSION

The data here presented show that in A. tigrinum females of the Chicago strain carrying an ectopic testis, the ovaries became transformed to testes in a high percentage of cases. The mode of reversal was comparable to that previously described by the writer ('29, '31 a, '36): inhibition of cortical growth in an ovary under the influence of a graft testis was followed sooner or later by proliferation of germ cells in its hilar or medullary region to form testicular lobules and thus convert the ovary itself into a testis, in at least a part of its extent.

The removal of the graft testis appeared to favor the hypertrophy of such testicular regions as had developed in the ovaries under its influence and to stimulate the development of lobules in additional regions. This is illustrated by animals nos. 406, 431 and 402 (figs. 4, 5 and 6), in which a progressive increase in testicular bulk is to be noted. The continued influence of the graft testis in no. 455, on the other hand, was probably a very important factor in preventing any marked increase in bulk in the testicular lobe known to have been present 5 months before autopsy (fig. 7). The writer ('37) has accumulated considerable evidence that one testis may inhibit another, this inhibition being especially noticeable when the testis of a rapidly growing species (as A. tigrinum) acts upon a testis less advanced in development (as that of A. punctatum). In the females of the present experiment, the ovary, after degeneration of its cortex, was essentially a testis rudiment, and probably subject to a similar inhibition from the large ectopic testis; if it gave rise to definitive testicular structures, it is probable that it did so despite the associated testis and not because of any stimulative induction exerted by that organ. An interesting case described by Witschi ('37) appears to illustrate in parabiotic twins the type of inhibition mentioned above. In this tigrinum-axolotl pair the ovaries of the axolotl underwent reversal, but gave rise to testes which at 4 years were very small and for the most part, non-functional. If we may judge from the results obtained in the present experiment, castration of the tigrinum co-twin would have permitted the reversed gonads of its partner to develop to a size approximating that normal for an axolotl male (see protocols of nos. 431 and 402).

That the graft testis cannot be removed before a certain time without risking cortical (ovarian) regeneration in the atrophic ovary is well shown by no. 495, in which the cortex quickly hypertrophied despite the presence in the ovary of a small area in which testis lobules had developed. A similar cortical regeneration often followed removal of the testis in the writer's earlier experiments ('31 b, '31 c), in which the operation was performed before the age of 9 months.

If such regeneration is to be avoided, especially in ovaries in which no considerable areas of reversal have yet appeared, removal of the graft testis must be postponed until the cortex has been rendered sterile or almost nearly so. A complete sterility of the cortex, in the present experiment, was found in animals of 8, 12 and 18 months in whose ovaries no reversal (lobule development) had yet occurred; in similar animals of 9 and 14 months cortical gonia were very few in number. It is probable, therefore, that if removal of the graft testis were delayed until the age of 15 or 16 months, the ovarian cortex would have become completely sterile in almost all instances. The hilar or medullary region of the ovary, which seldom lacks germ cells in this strain, would then be able to develop as a testis without the interference resulting from rapid cortical regeneration. Should this region have become sterile as well as the cortex, it is probable that the gonad would continue in this state indefinitely.

The method of ectopic implantation described in this report precludes the possibility of intermixture of graft and host tissues in the fashion suggested by Witschi ('32, '33) for orthotopic transplants. The graft testis at most comes into relation only with the cephalic end of the homolateral ovary of the host. The remainder of this gonad, and the contralateral ovary as well, cannot by any possibility be regarded as mosaics of donor and host tissues. When testicular lobules appear in these ovaries, therefore, they must arise through hypertrophy of the medullary component of the gonad and not from elements contributed by the graft.

If intermixture of gonadal tissues of graft and host actually occurs with any great frequency after orthotopic implantation, the results obtained by that method should differ markedly from those obtained in the present experiment, in which such intermixture has been largely or entirely precluded by ectopic grafting. It is therefore of considerable interest to compare the results secured by the two methods when using animals of the same species and strain.

A group of orthotopically grafted animals previously described by the writer ('31 c) is quite comparable as to ages at autopsy to the animals of the present experiment, and may be used for comparison with them. This group, incidentally, shows the highest incidence of supposed reversal of females found in any orthotopically grafted animals of this strain so far reported (48%).

While there were nine animals out of fourteen (64%) in the present experiment showing reversal as compared with 48% in the group mentioned above, allowance probably should be made for the fact that these ectopically grafted animals have two ovaries while the orthotopically grafted have but one. In the present experiment, on this basis, we have a reversal in fourteen of a possible twenty-eight ovaries, an incidence of 50%. Even if we omit no. 414, about which some question exists (see protocol), the incidence of reversal in the remaining animals is still 50% as compared with 48% in the orthotopic series. There is thus no significant difference between the two groups in total percentage of ovaries which ultimately showed reversal. If intermixture of graft and host gonads had occurred in the orthotopically grafted animals, the incidence of the supposed reversal in that group should have been markedly higher than that in the animals with ectopic grafts.

Although the animals of the orthotopic group showed an earlier reversal than the animals of the present experiment it is questionable whether this has great significance. The animals with ectopic grafts were retarded somewhat by exploratory operations at 3 and 5 months, while those of the orthotopic group were not subjected to exploration until they were considerably older. The two groups, in addition, were not given identical care, and the ectopic group suffered a certain retardation in development from this factor. An ideal comparison of the two methods of grafting would, of course, require that animals be grafted by each at the same time and then reared together under identical conditions; this is now being done with A. tigrinum of two other strains.

In view of the great similarity of the results already obtained by the two methods, it is difficult to believe that the findings after orthotopic grafting can be explained as due solely to intermixture of gonadal components of graft and host, a factor which is rather completely ruled out by ectopic implantation. The results obtained by the latter method and by parabiosis (Humphrey, '36) should in any event remove all doubt as to the possibility of sex reversal in the A. tigrinum female.

## SUMMARY AND CONCLUSIONS

1. Ectopic transplants of the gonad preprimordium of Amblystoma into the territory of somites 4 to 7, inclusive (pronephric region), give rise to gonads in almost all instances. Transplantation into this site largely or entirely precludes the intermixture of gonadal components of graft and recipient, since from its position the graft comes into relation, at most, only with the cranial end of the homolateral gonad of the host.

2. In fifty-two A. tigrinum of the Chicago strain, after ectopic transplantation as above, the sex type of host and graft gonads was found to be 11 ♂♂, 15 ♂♀, 14 ♀♂, and 12 ♀♀, the small symbol being used in each case to indicate the graft.

3. The females carrying testis grafts were identified as such by exploratory operations at 70 to 90 days; the development of testicular lobules in their ovaries was followed by successive explorations at more advanced ages. Nine of the fourteen (64%) finally showed varying numbers of lobules (seminal tubules) in one or both ovaries.

4. Removal of the graft testis after reversal had begun in any ovary was usually followed by hypertrophy of the area already showing lobules, and by the appearance of these structures in other regions.

5. The five animals which showed no testicular lobules in their ovaries at autopsy nevertheless had medullary germ cells (spermatogonia) present in considerable numbers. An inhibitory action of the graft upon this medullary (testicular) rudiment is suggested as an important factor in delaying its hypertrophy into a definitive testis.

6. From the trend toward cortical sterility observed in the present experiment it is concluded that removal of the graft testis, if postponed until the age of 15 months or later, would

generally permit ovaries not yet showing testicular development to do so unhindered by cortical regeneration. The medullary rudiment (potential testis) is seldom completely sterile in this species and strain.

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# PLATE 1

## EXPLANATION OF FIGURES

All figures on this plate are photographs (× 3) showing the gonads in situ, as exposed by removal of the gastro-intestinal tract, liver, etc. Since the cephalic end is upmost, the right side lies at the reader's left. See protocols (p. 458) for more complete record of any animal.

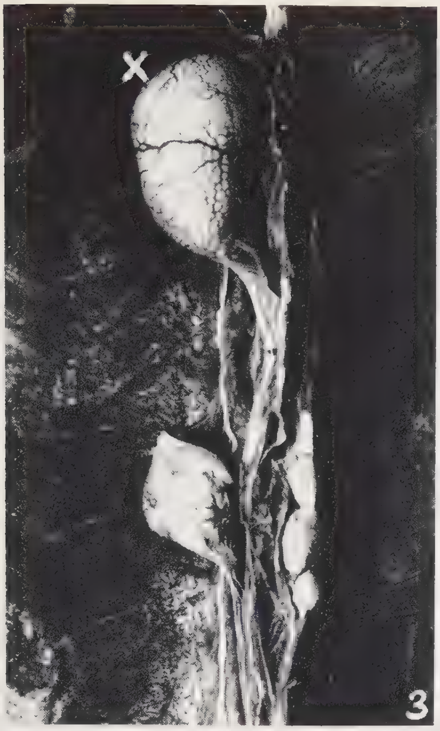
3 No. 441, age 11 months. The large graft testis (X) is well cephalad in position. Reversal has occurred in both ovaries, but the testicular lobules in the left are of more recent origin (note smaller size).

4 No. 406, age 16 months. The graft testis was removed at 11 months. The left ovary has been replaced by several testis lobes, only one of which was present on exploration at 9 months, while a single lobe has developed in the right ovary.

5 No. 431, age 16 months. The graft testis was removed at 10.5 months. These gonads (now large testes) were atrophic ovaries on exploration at 3 and 5 months, but reversal had begun before exploration at 9 months.

6 No. 402, age 19 months. The graft testis was removed 8 months previous to autopsy. The left ovary (at right) showed no reversal at 3 months, but a possible mass of small lobules at 5 months. A large part of the testicular tissue in this gonad, as well as all that in the right ovary, developed after removal of the graft.

7 No. 455, age 16 months. Graft testis at X. The left ovary has a testis lobe (arrow) which was present 5 months previously, but which has remained small, possibly due to the influence of the graft. The right ovary has a testicular lobe developed at its cephalic end (just caudal to the graft testis) as well as a very small lobe at its opposite end.



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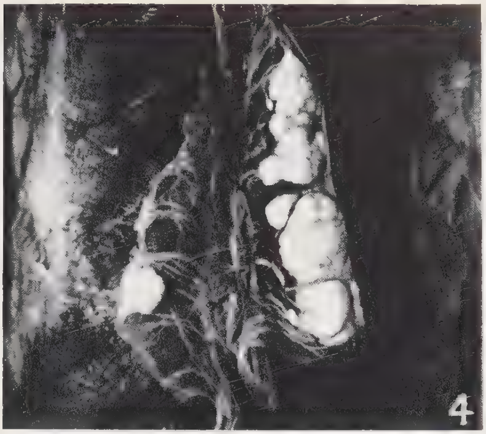

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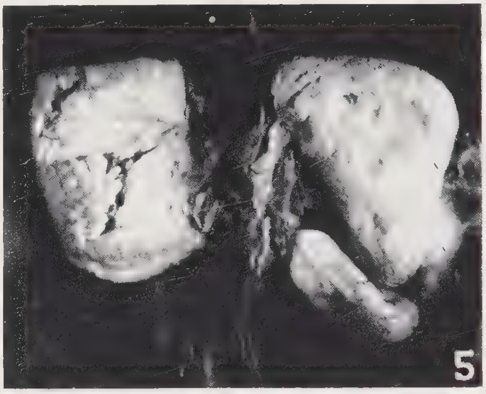

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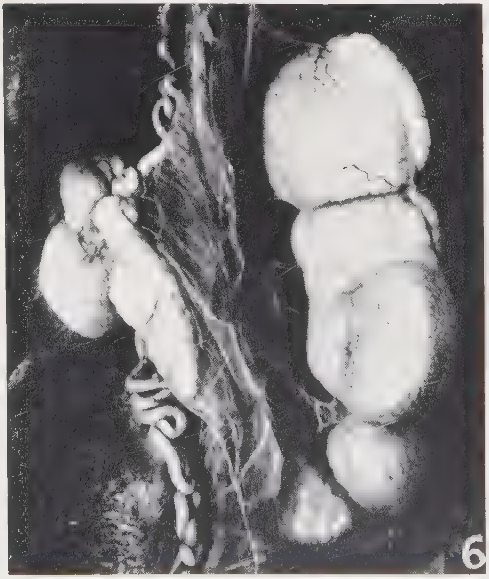

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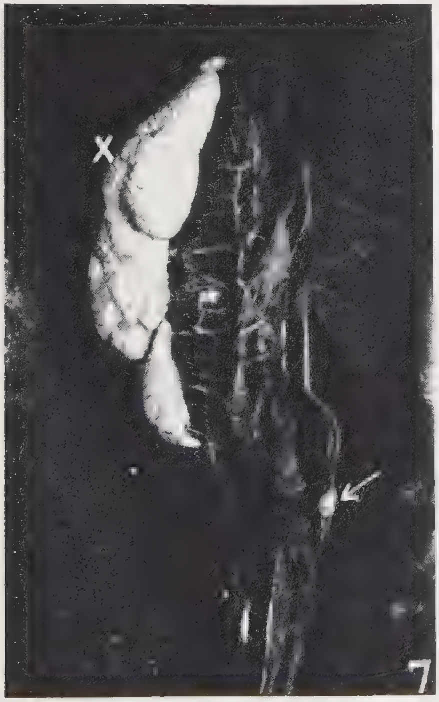

7

# THE OVUM OF THE MINK (MUSTELA VISON)

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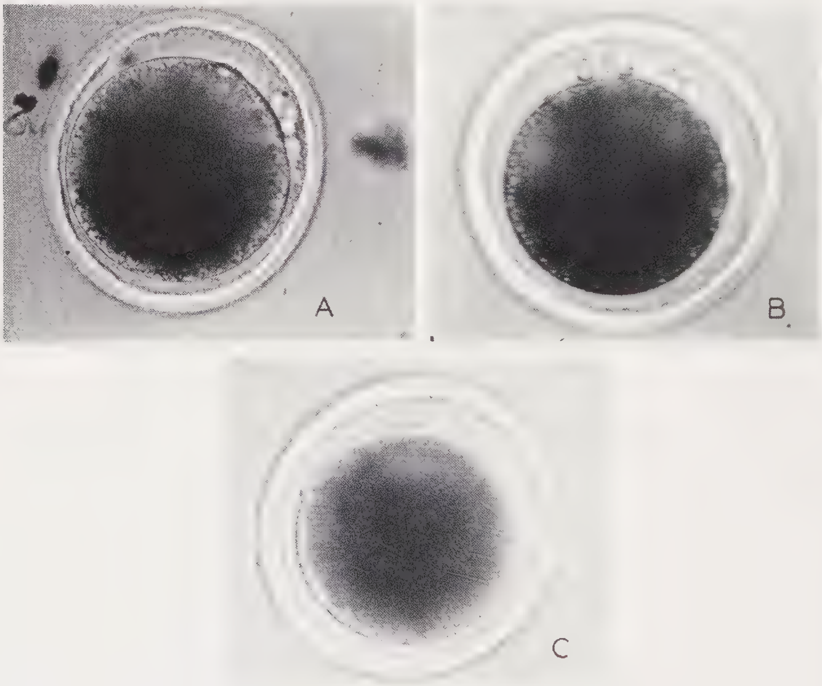
THREE FIGURES

As part of a cooperative study of reproduction in the mink (Mustela vison), made by the Biological Survey and the Carnegie Institution of Washington, and financed by Bankhead-Jones Cooperative Research funds, three tubal ova were recovered and photographed in living condition. The mink from which these were obtained was 3 years old. She had refused to mate during her first breeding season, and though she had mated once during her second year, she produced no young. This is not an unusual record, for minks appear to be far less prolific either in captivity or in the wild than one might expect from a knowledge of the common carnivores. The history of this animal is known, for she was bred at the United States Fur Animal Experiment Station, maintained by the Biological Survey at Saratoga Springs, New York.

During her third season she was placed with a male or males daily from March 9th until March 19th. Vaginal smears were made daily or oftener throughout this period. The right ovary, the uterine tube, and the uterine horn were removed under Nembutal anesthesia on March 10th. Nine days later live sperms were recovered from the vagina. Fifty-two hours after copulation, again under Nembutal, the left ovary, uterine tube, and uterine horn were removed. The uterine tube could be seen passing over the greatly distended capsule that enclosed the ovary. This condition had developed since the first laparotomy.

The ovum was free of follicle cells. The zona pellucida was sharply defined, relatively narrow. In egg A (see accompanying illustration) the zona appeared to be divided into three parts, which photographed with considerable distinctness, possibly due more to optical qualities than to distinctive structure. Egg C showed only a slight differentiation in the zona, while egg B was like C at a point opposite the dark bodies and like A elsewhere. The egg proper, or vitellus, did not fill the zona pellucida. Each egg had a perivitelline space filled with granules, a condition that appears in all mammalian eggs soon after fertilization.



Figures A, B and C

The cytoplasmic mass of egg A seemed to be connected to the zona by a single protoplasmic strand; otherwise the mass was free within the lumen.

In common with the ova of carnivores generally, so far as known, the cell mass is very dark because globules of lipoid material are distributed throughout the cytoplasm. If the surface of the mass is brought into focus the closely packed globules are easily observed. The larger granules that lie at the periphery measure about 3 μ in diameter. There is no tendency toward polar accumulation. Two dark bodies appeared in egg A at a tangential focus; similar bodies were even more clearly seen in egg B. What they represented was not determined, though they might be polar bodies. In all probability the eggs had been fertilized, for sperm heads were observed in each. While the age of these eggs cannot be stated, for the processes of ovulation and fertilization in the mink are not known, studies indicate that they were about 16 hours old.

TABLE 1

*Measurements of mink ova*

| OVUM | TOTAL DIAMETER | DIAMETER OF CYTOPLASMIC MASS: VITELLUS | THICKNESS OF ZONA |
|---|---|---|---|
| A | 0.135 × 0.148 | 0.11 | 0.011 |
| B | 0.151 | 0.109 | 0.011 |
| C | 0.143 | 0.103 | 0.0113 |

Many thanks are due to Dr. Carl G. Hartman and Dr. Warren Lewis, of Carnegie Institution of Washington at Johns Hopkins University, Baltimore, Md., for their very active aid in making these eggs available for study.

# STUDIES ON THE ENDOCRINES OF REPTILES

## I. THE MORPHOLOGY OF THE PITUITARY GLAND OF THE LIZARD (ANOLIS CAROLINENSIS) WITH SPECIAL REFERENCE TO CERTAIN CELL TYPES

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TWO PLATES (ELEVEN FIGURES)

### INTRODUCTION

Some characteristics of certain members of the reptilian group present problems of interest which fit in with the current fields of endocrinological investigations. Hypophyseal-gonadal relationships and a possible pituitary-chromatophore mechanism are two such problems. These have been investigated from the physiological point of view, the former by Herlant ('33) in Lacerta and Anguis; Clausen ('35) in snakes; Turner ('35) in the lizard, Eumeces laticeps; Evans ('35 a and b) in Anolis carolinensis; Mellish ('36) in Phrynosoma cornutum; and Forbes ('37) in the alligator Kleinholz ('36) used Anolis carolinensis and Parker ('38), Phrynosoma, to investigate the pituitary-chromatophore mechanism. However, these investigations account for only one aspect of the problems involved.

There is another approach which has not been considered as fully as it might be, namely, the histological one. A careful examination of the microscopic anatomy of the structures involved in such problems would perhaps contribute much toward a more specific knowledge of, among other things, the role of the pituitary gland in reproduction and in skin pigmentation. Only two references concerning the cytological aspects of the pituitary gland in reptiles in relation to a specific physiological condition are available (Viguier, '11; Siler, '36). Both of these deal with the effects of thyroidectomy on pituitary cell types.

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[1] Accepted in partial fulfillment of the requirements for the degree of Master of Science at New York University.

As a preliminary to future experimental investigations involving changes in the gland, it is important to have a thorough knowledge of the normal anatomy and histology of the form to be used. Charipper ('37) stresses this from the point of view of comparative anatomy and physiology. He believes that "It may lead us to a more sober attitude in our interpretations."

The purpose of this paper, therefore, is to describe the normal anatomy and histology of the pituitary gland of Anolis carolinensis as a basis for future experimental research.

The writers are indebted to Dr. G. K. Noble of the American Museum of Natural History for some of the animals utilized in the initial stages of this investigation.

## MATERIAL AND METHODS

Anolis carolinensis is a species of iguanid lizard found in the southern United States. It is commonly referred to as the American 'chameleon.' The forms used in this study were obtained from Thibodaux, Louisiana, at varying intervals extending over a period from April to March. The animals survived well in the laboratory where they were kept in a glass terrarium with a screen top. During the summer months they were fed on houseflies but during the remainder of the year when flies were not available, mealworms (Tenebrio molitor) served as an excellent substitute. The terrarium was sprinkled twice daily for the purpose of supplying drinking water.

The animals used for histological study ranged from 50 mm. to 60 mm. in length. Measurements were made from the tip of the head to the cloacal slit. The entire head was removed and trimmed off just posterior to the pituitary gland which could easily be seen through the bone. The lower jaw was cut off and the remainder of the head immediately dropped into fixative. Helly's, Bouin's, and Carnoy-Lebrun were used as fixing agents. Carnoy-Lebrun proved to be an excellent fixative since it penetrated very rapidly inhibiting any postmortem changes. The majority of heads were fixed in the latter solution. As little as 6 hours fixation was found to work well. The heads were decalcified in phloroglucin after which the bone of the roof of the skull was removed. The basisphenoid bone was left intact in order not to disturb the normal position of the gland in relation to the brain and surrounding structures. Several changes of dioxan served to completely dehydrate and clear the heads. Cross, median sagittal, and horizontal serial sections were made. All sections were cut at 5 μ.

For a study of the general anatomical features of the gland the slides were stained in Harris' hematoxylin and eosin. For cellular differentiation the Masson stain was used. In the latter process the slides were placed into xylol and run down the alcohols to distilled water. They were put into Masson A (acid fuchsin) for 2 minutes, differentiated in Masson B (phosphomolybdic acid) for 4 hours, and then placed into Masson C (aniline blue) for 1 to 1½ hours. The slides were washed in distilled water and run quickly through 95% and absolute alcohol to xylol. They were mounted in gum damar.

## DESCRIPTION

### *A. Relation to surrounding structures*

The pituitary body of Anolis carolinensis lies in a slight depression of the basisphenoid bone in the region between the basipterygoid processes. The anterior portion of the gland is bounded ventrally by the presphenoid cartilage which extends forward from the basisphenoid bone. Posteriorly this bone arches over the pituitary to completely surround the caudal extremity of the gland and form a ledge between it and the brain. These relationships are readily seen in a median sagittal section through the brain and pituitary gland (fig. 2). An extension of the dura mater continues into the sella turcica covering the pituitary ventrally and laterally and giving off a partition which separates the pars anterior from the pars intermedia except at the posterior end where these two parts of the gland are connected. The subarachnoid space is continuous down the stalk and around the pars anterior and intermedia giving in effect a vascular cavity in which the gland rests. Extending from the lateral ventral region of the gland on both sides are striated muscle masses which are directed anterolaterally and seem to be continuous with the muscles of the eyes. They are best seen in a cross section of the gland (fig. 4).

The pituitary is attached at its anterior end to the floor of the diencephalon by a relatively long thin-walled infundibular stalk which is directed posteriorly and slightly ventrally. The infundibular cavity is bounded ventrally by the thickened lamina postoptica. The cavity of the third ventricle is continuous with the large infundibular cavity which narrows down posteriorly to form the infundibular recess (fig. 1 d).

The cerebral carotid arteries run lateral to the pars intermedia (fig. 3). A large thin-walled blood sinus is present dorsal to the gland in the caudal region (fig. 3). No blood connections could be traced from these vessels to the pituitary gland. Blood capillaries, however, enter the gland with the connective tissue from the surrounding membranes.

### *B. Gross anatomy*

The pituitary of Anolis consists of three lobes—the pars anterior, the pars intermedia, and the pars nervosa. There is no evidence of a pars tuberalis. The pars anterior is the most ventral portion of the gland. It is an elongate structure with a broad caudal end which gradually decreases in width up to a distance about one-third of the length from the posterior end. At this point there occurs a gradual increase in width toward the anterior extremity where the lobe ends in a blunt point under the lamina postoptica. The shape is best observed in the ventral view shown in figure 1 c. Two small anteriorly directed finger-like projections originate on either side of the broad caudal end of the pars anterior. They terminate at about the level of the constriction in the anterior lobe. The pars anterior is the longest of the lobes, varying in length from 642.5 μ in a 50-mm. specimen to 1250 μ in a 65-mm. specimen. In cross sectional views one can observe a distinct change in shape from the anterior to the posterior extremities. The cranial end is more or less triangular with the broad base of the triangle dorsally situated (fig. 4). In the constricted portion of the lobe an elliptical shape is assumed (fig. 5), this being retained almost to the extreme posterior end where it takes the form of an oval (fig. 3).

The pars anterior is attached only at its posterior end by a narrow strand of cells to the caudal extremity of the pars intermedia which lies directly dorsal (fig. 1 d). The pars intermedia is a broad more or less rounded structure and is greater in volume than any other part of the gland. Ventrally it forms a concavity into which the elliptical and oval regions of the pars anterior fit (figs. 3 and 5). The intermedia is deepest at about the mid-region (fig. 5). It narrows down posteriorly to form a flattened, broad plate of cells. At this region there appear laterally two rather deep grooves which run at an angle posteriorly and ventrally toward the mid-line (fig. 1 c). It is in these grooves that the cerebral carotid arteries run (fig. 3).

Anteriorly the pars intermedia is closely applied to the pars nervosa. The intermedia surrounds the nervosa laterally but not dorsally and ventrally except at the posterior end. Irregular processes extend posteriorly from the dorsal part of the nervosa and interdigitate with processes from the pars intermedia (fig. 1 a). The infundibular cavity penetrates the pars nervosa sending hollow diverticula into the posterior processes. In a horiontal section through the dorsal part of the gland, the system of branching cavities is quite evident (fig. 8). In cross section the pars nervosa appears to be made up of distinct areas separated by dorsoventral connective tissue septa, each area containing within it a branch of the infundibular cavity (fig. 4).

### *C. Microscopic anatomy*

*1. Pars anterior.* The cells in the anterior lobe are arranged in anastomosing and branching cell cords which run in an anterior-posterior direction. These cords are usually two cells in thickness and are separated by large capillary channels (fig. 6). In cross section the pars anterior has an aciniform appearance due to the grouping of the cells about the blood capillaries (fig. 11). In the posterior region of the lobe there is a decided enlargement of the capillaries to form sinuses (fig. 6). In some regions the vascular lumina may be seen to be continuous with the peripheral blood sinus which appears to surround the lobe.

The cell types differ in the anterior and posterior regions of the gland. Anteriorly there are, bordering on the capillaries, columnar cells with a basal, spherical nucleus, and fine, densely packed eosinophilic granules gathered on the side between the nucleus and the apex of the cell (fig. 6). The nucleus is vesicular in nature, and contains one eosinophilic nucleolus. Also bordering on the capillaries are taller columnar cells with basal vesicular nuclei and large blue-staining granules which are more diffuse than those of the eosinophilic cells. Between the bases of the columnar cells are a few round cells with an intensely eosinophilic coarsely granular cytoplasm and a spherical nucleus displaced to one side of the cell. The major proportion of the cells in this region, however, are round cells with an extremely large nucleus and a small amount of cytoplasm. Cell outlines are not very distinct. A few basophilic granules are present.

Lining the blood sinuses in the posterior part of the gland are large round to ovoid cells which take an homogeneous eosinophilic stain (fig. 9). The basally situated nucleus has an irregular spherical shape and in many cases a crescent shape is obvious. Similarly staining cells are present between the sinuses. In these elements the nucleus is displaced to one side of the cytoplasm. Large round basophiles similar to those in the anterior region occupy the remaining area between the sinuses. They are especially abundant in the ventral portion of the lobe where they also border on the sinsues and assume a columnar shape. A few columnar granular eosinophiles, characteristic of the anterior region of the lobe, may also be seen bordering on the sinuses.

*2. Pars intermedia.* The pars intermedia in horizontal and cross sections consists of cords of cells arranged about fine blood capillaries. The cords run in an anteroposterior direction and make up the mass of this portion of the gland (fig. 10). Here again, as in the pars anterior, the cavities of the blood vessels may in places be seen to be continuous with the lumen of the enveloping vascular sinus. The majority of blood elements, however, enter with the connective tissue extensions from the membrane surrounding the pars nervosa. The cells making up the cords are very tall columnar with small basal spherical nuclei. These nuclei, like those of the anterior lobe, are vesicular and have an eosinophilic nucleolus. The cytoplasm is filled with fine and coarse reddish purple granules. Between the bases of the columnar cells are large, somewhat oval cells with rather irregularly shaped nuclei (fig. 10). These cells contain a homogeneous reddish orange cytoplasm in which may be observed a few small clear vacuoles. Somewhat lighter staining accumulations of an amorphous material are also present between the bases of the cells. Vacuoles of various sizes are present in this colloid-like material (fig. 7).

*3. Pars nervosa.* The pars nervosa consists entirely of a rather compact fibrous tissue with a few scattered oval to spherical nuclei. This tissue ramifies into the pars intermedia, not as a solid mass, however, but penetrated by hollow diverticula of the infundibulum. A single row of typical ependymal cells lines the cavities thus formed. The nervosa is supplied with blood vessels from the connective tissue surrounding the lobe. No colloid accumulations were evident in the sections studied.

## DISCUSSION

The relationship of the pituitary gland in Anolis to the surrounding structures in the skull is similar to that described in other reptiles. Tilney ('11) and Stendell ('14) speak of the pituitary as lying in a deep sella turcica. Siler ('36) states that the sella turcica in which the gland of Thamnophis lies is a depression in the basisphenoid bone. Anolis similarly lies in a depression in the basisphenoid bone and resembles the turtles, Chrysemys elegans and Pseudemys picta (Baumgartner, '16) and the garter snake, Thamnophis radix (Siler, '36) by being completely surrounded caudally by a dorsal extension of the basisphenoid. Like Sphenodon it is bounded ventrally at its anterior end by the presphenoid cartilage. Staderini ('03) called attention to two striated muscle masses at the sides of the pituitary in Gongylus. Gisi ('07) noted them in Lacerta; Baumgartner ('16) in Sceloporus, Chrysemys, and Pseudemys; and Wyeth and Row ('23) in Sphenodon. The latter investigator refers to them as the internal pterygoid muscles. Baumgartner merely suggests that they probably represent eye muscles. The observations on Anolis confirm this since they appear to be directly continuous with the ocular muscles. The cerebral carotid arteries have been described as running alongside the caudal end of the pituitary in Lacerta, Chrysemys, Pseudemys, Sceloporus, Alligator (Baumgartner, '16) and Sphenodon (Wyeth and Row, '23). These have also been observed in Anolis. No blood vessels could be traced from these vessels into the gland. Baumgartner ('16) also reports that an injected alligator showed no direct branches from the carotids to the hypophysis. Baumgartner noted a large venous channel, caudodorsal to the hypophysis in lizards and snakes, which he believes drains the organ. A large blood sinus, similar in position, was noted in Anolis. Whether the organ is drained by this channel remains to be determined. In this lizard definite capillary channels penetrate the gland from the investing arachoid membrane. This agrees with the observations of Siler ('36) on Thamnophis.

The posteriorly directed infundibular stalk seen in Anolis is characteristic of reptiles in general (de Beer, '26; Tilney, '11; Stendell, '14; Gentes, '07). de Beer describes a long slender stalk in the tortoise, grass snake, Lacerta and Sphenodon, and Stendell speaks of a long stalked infundibular process in the Lacertidae, Varanus, Scincus, Chamaeleon, Iguana, and Basiliscus. Anolis resembles these forms in regard to the length of the stalk and simulates Lacerta muralis and L. viridis in sending branching diverticula from the infundibular cavity into the nervosa (Stendell, '14; Gentes, '07). This arrangement has also been described by Stendell for Emys and by Herring ('13) for Testudo.

Anolis possesses the three lobes characteristic of all reptiles, namely, the pars anterior, pars intermedia, and pars nervosa. The ventral position of the pars anterior agrees with the descriptions of de Beer ('26), Tilney ('11), Sterzi ('04), Baumgartner ('16) and Siler ('36) on other reptilian forms. Anolis resembles other Sauropsids (Stendell, '14) in having the anterior lobe only loosely connected at its posterior end to the pars intermedia. In this respect it differs from the Chelonians (Gentes, '07) where the two lobes are joined at both anterior and posterior ends. de Beer ('26) describes in the tortoise a forward prolongation of the pars intermedia which he refers to as the pars tuberalis. In Cistuda and Alligator, Tilney ('11) speaks of an accessory tongue-like projection extending forward from the anterior extremity of the gland toward the chiasma. Baumgartner ('16) states that the lateral buds persist to form a pars tuberalis and cortical zone about the anterior lobe in turtles and a tongue-like process and spiral band about the anterior lobe in alligators. Stendell ('14) states that in some forms (Chelonia and Saurians) there is a long tongue-like process projecting from the pars anterior. Gentes ('07) remarked that there is no formation in lizards which corresponds to the lateral lobes of the pituitary. This observation is confirmed in the Anolis adult but whether it exists in the embryo as has been described for Lacerta muralis (de Beer, '26) is not known.

There is no evidence of an hypophyseal cavity in Anolis. This is not unusual in the reptilian group since the cavity persists in some forms and disappears in others. Stendell ('14), de Beer ('26), and Baumgartner ('16) describe a residual lumen in the pars intermedia of some lizards. Herring ('13), Gentes ('07) and de Beer report the presence of a cavity between the pars intermedia and the pars glandularis in Chelonians.

The cord-like arrangement of cells in the anterior lobe is peculiar to all reptilian forms that have been described and is also an outstanding feature of the Anolis pituitary. A great deal of variation, however, exists in the cell types. de Beer found very little difference between the cells apart from being eosinophilic or basophilic. Tilney ('11) described deeply staining and faintly staining acidophiles in the turtle as well as peripherally located basophiles. Herring ('13) reports the presence of clear non-granular cells and deeply staining granular elements in the tortoise. In the alligator Tilney finds acidophiles and basophiles while Baumgartner ('16) observed some cells that took eosin while others remained clear. In Eutaenia only faintly staining acidophiles are present (Tilney, '11). Siler ('36) observed three types of cells in Thamnophis—cells with coarse red-staining granules, cells with finer acidophilic granules which he considers to be chromophobes, and beta cells with fine granules. These obviously confusing results are very likely attributable to differences in technique, thus precluding any comparison of the work of these various investigators. Three types of cells are also present in Anolis—cells with eosinophilic granules, cells with basophilic granules, and non-granular chromophobes.

The anterior lobe in Anolis is extremely vascular and in this respect agrees with other reptiles with the exception of Eutaenia in which no blood vessels were observed (Tilney, '11). Anolis resembles Sphenodon in showing an increase in the size of the vascular sinuses toward the posterior end of the lobe. No colloid was noted in Anolis, such as has been described in the lumina of the cell cords in the turtle and alligator (Tilney, '11); tortoise (Herring, '13); and intercellularly in Emys and Cistuda (Stendell, '14).

The pars intermedia of Anolis is unusual because of its large size, being the largest lobe in the gland. Stieda (1875) found a small dorsal portion closely united to the lobus infundibuli and a large ventral lobe. Stendell ('14) observed a very small 'zwischenlappen' in Sauropsida and Chelonia but noted that it was fairly well developed in Ophidia. Herring, ('13) noted the presence of a well-marked pars intermedia in Testudo and Lacerta viridis. Gentes ('07), on the other hand, found a small intermedia in Lacerta viridis, L. muralis, and Cistudo. Most investigators noted that the intermedia had a poor staining reaction (de Beer, '26; Tilney, '11; Stendell, '14; Gentes, '07). The well-defined, intensely staining granular cytoplasm of Anolis is thus in contrast to what has been noted in other forms. Concerning the blood supply, de Beer ('26), Stendell ('14), and Gentes ('07) report that this lobe is sparsely supplied with blood vessels. Tilney ('11) found no blood vessels at all in Eutaenia, and Stendell ('14) states that the intermedia of sauropsids lacks blood vessels entirely. In this respect too, the pars intermedia of Anolis is different from that of other reptiles, since it possesses a definite system of fine capillaries. On the basis of its large size, heavily granular cells, vascular supply, and colloid-like secretory material, the pars intermedia may be interpreted as a functionally active portion of the pituitary gland. Kleinholz ('36) has shown that the characteristic body color changes observed in Anolis are in part due to a pituitary hormone. Zondek ('35) has isolated a chromatophoric principle, 'intermedin,' from the pars intermedia of many forms. Further histological and cytological investigation of the intermediate lobe of Anolis under various conditions may offer evidence to substantiate the conclusions of other investigators that the pars intermedia produces an active principle which controls color changes. It is interesting to note that Zondek ('35) was able to demonstrate the hormone in the walls of the third ventricle and nowhere else, a fact which suggests the pituitary stalk as a pathway for the distribution of this hormone. The increased surface of the infundibular cavity in Anolis, brought about by the branching diverticula, might well be associated with such a function.

Anolis is thus seen to resemble the other members of the reptilian group in general anatomical features and cord-like arrangement of cells in both pars anterior and pars intermedia. The main difference exists in the features of the pars intermedia which have already been described.

## SUMMARY

1. The pituitary gland of Anolis carolinensis lies in the basisphenoid bone and is surrounded by the dura mater and arachnoid membrane from which blood capillaries enter the gland.

2. The short infundibular stalk is directed backward carrying the intermedia and nervosa posteriorly. The anterior lobe is ventral and is attached to the intermedia only at its posterior end. There is no pars tuberalis.

3. The infundibular cavity is continuous with the third ventricle and sends hollow diverticula into the nervosa. These cavities are lined with a single layer of ependyma cells.

4. The pars anterior is the longest part of the gland while the pars intermedia is the greatest in volume.

5. Three distinct cell types are present in the anterior lobe —eosinophilic and basophilic granular cells and pink-staining non-granular cells.

6. The pars intermedia consists of columnar cells with red-purple granules and oval cells with non-granular orange cytoplasm. Colloid-like accumulations are also present.

7. There is no evidence of a residual lumen.

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## PLATE 1

### EXPLANATION OF FIGURES

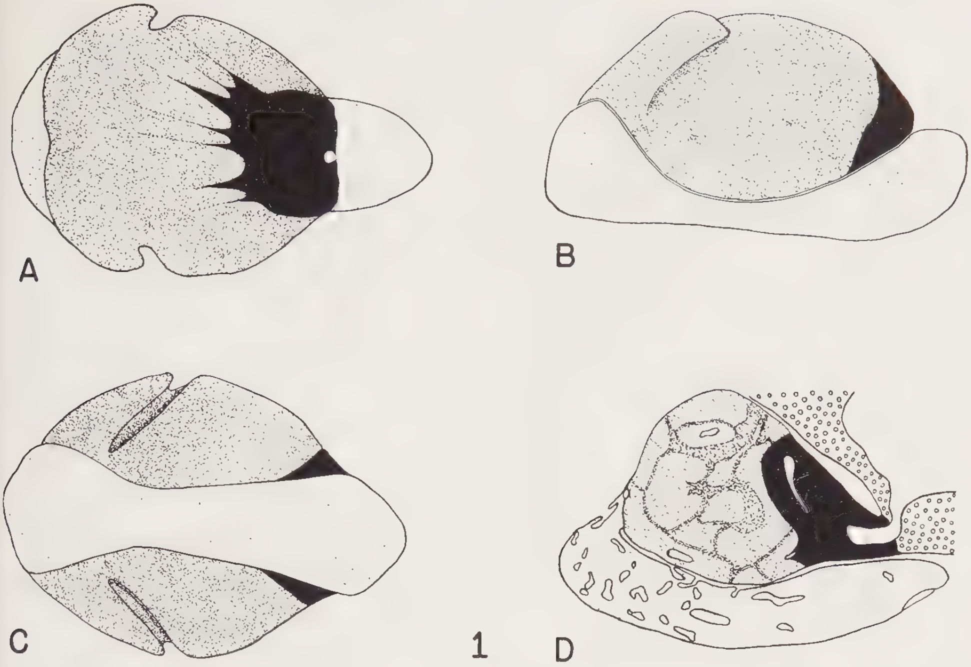
1 Drawings of the pituitary gland as seen from four different views, dorsal (A), lateral (B), ventral (C), and median sagittal (D). Pars anterior is coarsely stippled, pars intermedia finely stippled, and pars nervosa is solid black. Anterior is to the right. × 145.

2 Photomicrograph of a sagittal section through the brain and pituitary gland showing the arching of the basisphenoid bone over the gland, and the presphenoid cartilage extending forward from the bone. The optic chiasma is seen to the left in front of the gland. Carnoy-Lebrun, Masson. × 10.

3 Photomicrograph of a cross section through the posterior portion of the gland showing the two grooves on the sides of the pars intermedia in which run the cerebral carotid arteries. Dorsally situated is a large blood sinus. The pars anterior is ventral. Carnoy-Lebrun, Masson. × 120.

4 Photomicrograph of a cross section through the anterior portion of the gland showing the pars nervosa with four branches of the infundibulum. The pars intermedia is seen lateral to the nervosa. Note the dark muscle masses under the gland. Carnoy-Lebrun, Masson. × 120.

5 Photomicrograph of a cross section through the mid-region of the gland showing the large size of the intermedia and the cord-like arrangement of cells. Carnoy-Lebrun, Masson. × 120.



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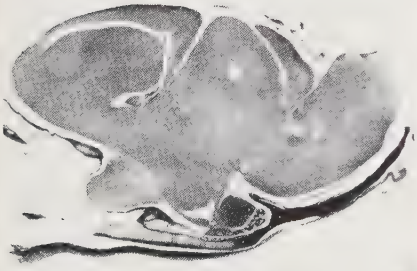

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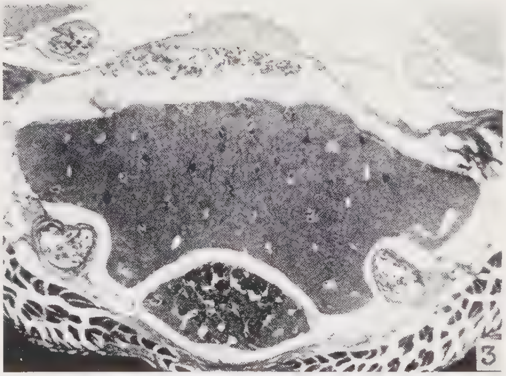

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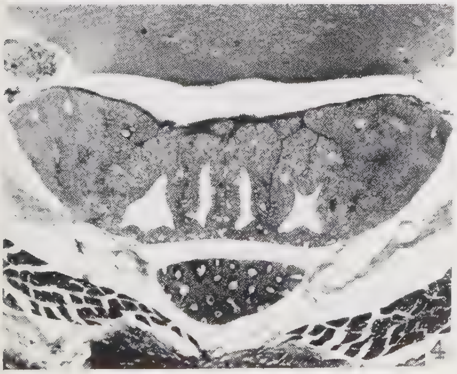

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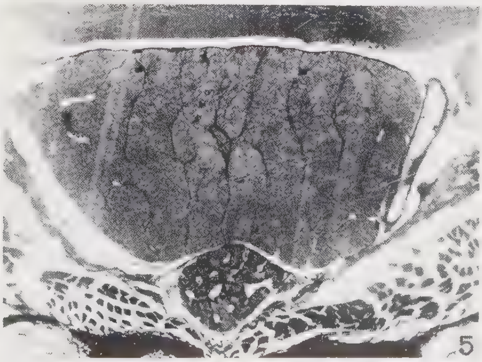

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## PLATE 2

### EXPLANATION OF FIGURES

All figures are photomicrographs of sections from glands fixed in Carnoy-Lebrun and stained with Masson.

6 Frontal section through the pars anterior showing the cords of cells separated by blood capillaries. The columnar granular cells on the right are mostly eosinophiles. The dark cells on the left are the non-granular pink staining cells characteristic of the posterior end of the lobe. × 600.

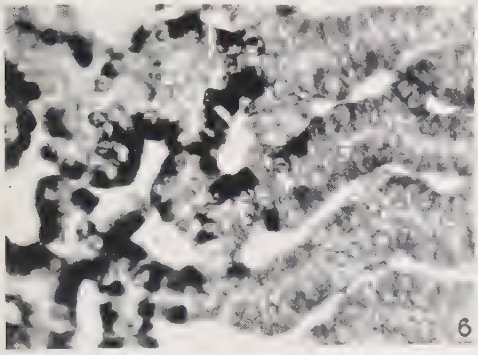
7 Section through the pars intermedia showing colloid-like accumulations between the bases of the columnar cells. × 600.

8 Frontal section through the gland showing the branching diverticula in the nervosa. The pars intermedia is seen closely applied to the nervosa. × 120.

9 Cross section of the posterior end of the pars anterior. The large dark cells bordering on the sinuses are the same as those in figure 6. The lighter staining cells on the left are basophiles. × 1400.

10 Section through the pars intermedia showing the arrangement of cells. Note the small dark cells between the bases of the columnar cells. These have a homogeneous reddish orange cytoplasm. × 600.

11 Cross section through the anterior lobe showing the numerous blood sinuses. × 600.



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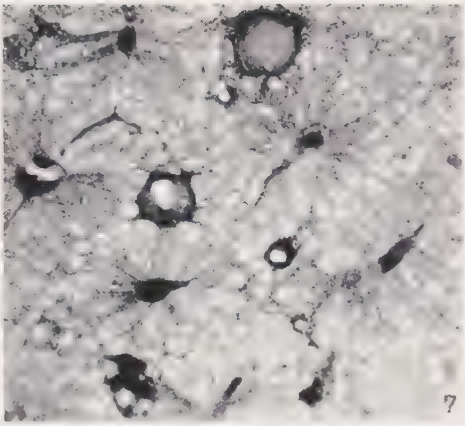

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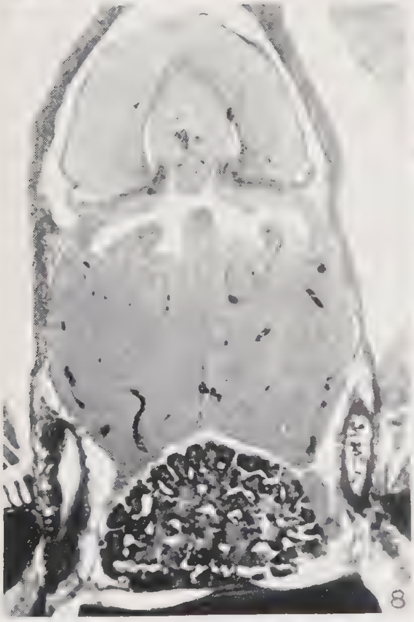

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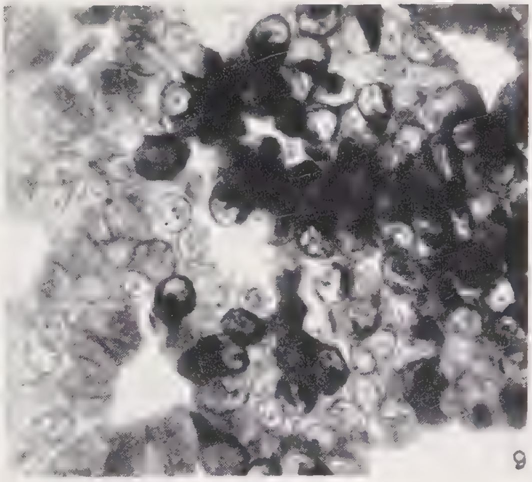

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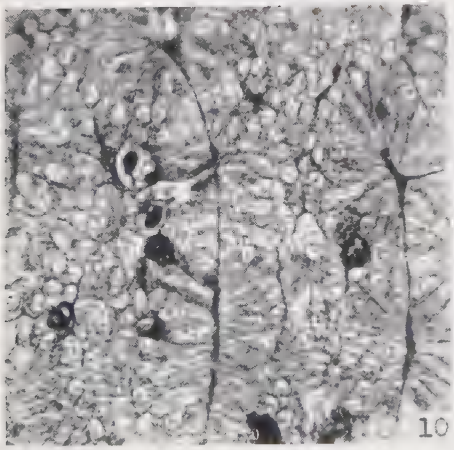

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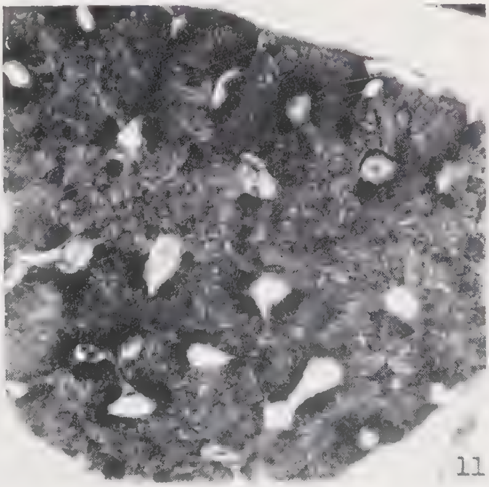

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# STIMULATION OF THE THYROID GLAND OF THE GUINEA PIG BY ANURAN ANTERIOR PITUITARY[1]

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ONE PLATE (FOUR FIGURES)

Recently, Adams and Tukey ('38) observed activation of the thyroid gland of the mouse following injections of pars anterior of Rana pipiens. It was therefore thought worthwhile to examine the thyroid of the guinea pig after administration of anterior pituitary substance from the same species of frog that caused a positive reaction in mice, especially since the thyroid gland of the former is reputedly more sensitive to mammalian thyrotropic hormone than that of the latter (Loeb, Saxton and Hayward, '36)[2] and since the attempts of Houssay, Novelli and Sammartino ('32) and Magdalena ('32) to activate the guinea pig thyroid with anuran (toad) pars anterior had produced slight or doubtful reactions. It was found that eight or sixteen anterior pituitaries of Rana pipiens given in 4 days caused a definite stimulation of the thyroid of the guinea pig.

## MATERIALS AND METHODS

Guinea pigs 15 or 16 days old at the beginning of treatment were used for the tests. They were injected daily for 4 days

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[1] Aided by a grant from the Rockefeller Foundation.

[2] Aron, Loeb and their co-workers (Aron, '29, '30 a, b; Loeb and Bassett, '29, '30) showed that the thyroid gland of the guinea pig was very sensitive to the hormones contained in the anterior pituitaries of cattle. Since 1929 and 1930 largely in continued work of Aron and Loeb it has been used as a test object for thyrotropic hormone in the anterior lobes of pigs, sheep, rabbits, rats, cats, dogs, horses and humans as well as in blood, serum, cerebro-spinal fluid and urine of several of these mammals.

with two or four fresh anterior lobes of female Rana pipiens,[3] a total dosage of eight or sixteen glands per animal, and were autopsied on the fifth day. The anterior lobes were removed from decapitated frogs, weighed, macerated in 0.3 cc. of 0.9% NaCl solution and injected into one of the hind legs of the animal. Controls received frog brain tissue. Thyroids were preserved in Helly's fluid, sectioned at 5, 7.5 and 10 μ, and stained in Mallory's connective tissue stain or in haematoxylin and eosin.

TABLE 1

*Data re thyroid weights of guinea pigs 3 weeks old at autopsy after injections of fresh pars anterior and brain tissue of Rana pipiens*

EXPERIMENTAL

| Animal | Total dosage frog P. A. | | Body weight | | Thyroid weight | |
|---|---|---|---|---|---|---|
| | Number | Weight | Initial | Autopsy | Absolute | Per cent of body weight |
| | | *mg.* | *gm.* | *gm.* | | |
| 1 ♀ | 16 | 22.4 | 126.40 | 124.20 | 18.4 | 0.015 |
| 2 ♀ | 16 | 19.7 | 113.00 | 125.35 | 36.7 | 0.029 |
| 3 ♂ | 16 | 17.8 | 138.00 | 148.60 | 30.1 | 0.020 |
| 4 ♀ | 16 | 17.4 | 107.80 | 109.10 | 44.6 | 0.041 |
| Average (4) | | 19.3 | 121.30 | 126.81 | 32.5 | 0.026 |
| 5 ♀ | 8 | 10.3 | 82.00 | 84.62 | 19.6 | 0.023 |
| 6 ♀ | 8 | 7.7 | 83.10 | 106.40 | 28.5 | 0.027 |
| 7 ♂ | 8 | 8.3 | 93.90 | 101.80 | 25.4 | 0.025 |
| Average (3) | | 8.8 | 86.33 | 97.61 | 24.5 | 0.025 |
| Average (7) | | 14.8 | 106.31 | 114.30 | 29.0 | 0.026 |

CONTROL

| | Total dosage frog brain | | | | | |
|---|---|---|---|---|---|---|
| | | *mg.* | | | | |
| 8 ♂ | | 13.7 | 123.30 | 129.70 | 13.4 | 0.010 |
| 9 ♀ | | 7.0 | 121.40 | 129.70 | 16.0 | 0.012 |
| Average (2) | | 10.4 | 122.35 | 129.70 | 14.7 | 0.011 |

OBSERVATIONS

Table 1 contains the data concerning the seven guinea pigs tested and their two controls. An increase in absolute thyroid weight and in its percentage of body weight over that of the controls occurred in every animal that received frog pars anterior, but in one (no. 1) the increase was slight. The average increase in absolute weight for those animals given sixteen glands was 121% and for those with eight glands, 66%. Stimulation was apparent in the histological picture in the thyroids of all of the animals given frog anterior pituitary substance, while the thyroids of controls remained in a resting condition (figs. 1 to 4). The follicular epithelium was higher; the colloid less abundant and less homogeneous, its marginal vacuoles more numerous and larger, and in Mallory's stain, it was blue in contrast to the bright orange of the control glands. The follicles were usually more active in the center of the gland, though the margin of less active ones at the periphery was typically not more than one row in width. Mitoses were not abundant but exceeded the number in control glands.

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[3] These experiments were carried on in March, so that the pituitaries were from females in the pre-breeding season.

## DISCUSSION

It is evident from these results that frog (Rana pipiens) anterior pituitary substance in the amounts used increases the weight and stimulates the cells of the guinea pig thyroid. The conclusion that this stimulation is actually due to anterior pituitary hormones and not to the proteins of the amphibian tissue seems justified since frog brain caused no such reaction in controls. The activation in the experimental animals presents a contrast to the findings of Houssay et al. ('32) and Magdalena ('32) who observed a slight or doubtful reaction of the guinea pig thyroid after doses of twenty toad anterior lobes or 'plusieurs dizaines' per animal. But it is impossible to make an exact comparison with their studies since details such as weight of toad pituitary substance injected, numbers of days of treatment, weight and age of guinea pig hosts and pictures of the glands are not given in their reports. From the context in Houssay's paper, however, it seems probable that the guinea pigs tested were heavier than those in the present experiment, since he and his co-workers were reporting on the effects of transplanting anterior lobes of normal and thyroidectomized rats, guinea pigs and dogs into guinea pigs whose controls weighed between 140 and 230 gm. and averaged 176 gm. Moreover in their tests the glands were transplanted into the body cavity for 2 days and the animals killed on the third day. Aron ('29) claims that young guinea pigs just after birth react to smaller doses of anterior lobe substance than do older ones. Thus it is possible that the difference in the results in the present experiment and in those of Houssay and Magdalena may be due to a greater sensitivity of the younger animal and also to a longer treatment. Furthermore it is conceivable that the toad's anterior lobe contains less thyrotropic hormone than the frog's. Loeb ('32) found such marked quantitative differences in mammalian anterior lobes that it was necessary to administer 44.5 mg. of fresh anterior lobe of the guinea pig to give a stimulus of that animal's thyroid equivalent to that secured with 3 mg. of rat or 4 to 6 mg. of rabbit anterior lobe.

It is interesting to compare the increases in absolute thyroid weight of the guinea pig, 121% for the four animals given sixteen glands and 66% for the three given eight glands, with the 74% increase in four mice given sixteen glands in 4 days with autopsy on the fifth (Adams and Tukey, '38). The average dosages of frog pars anterior that induced these increases are 19.3, 8.8 and 16.2 mg., respectively. Thus it appears that the mouse thyroid responds relatively less than that of the guinea pig to somewhat similar doses of frog anterior lobe. It is also less active when examined histologically. Some difficulty in activating the mouse thyroid with mammalian pars anterior has been reported by Loeb, Saxton and Hayward ('36) and by Adams and Tukey ('38).

It was thought that it would also be of interest to examine these weight increases and those induced in guinea pig thyroids by varying amounts of mammalian anterior lobe substance in order to compare the thyrotropic content of the anuran and mammalian pituitaries. However, no definite conclusions can be drawn from such comparisons. In some of the papers giving applicable data the guinea pig hosts weighed more than in the present experiment and the period of treatment differed. For example, Aron ('30 b) reports that single doses of extract containing the equivalent of 33 mg. and 330 mg. of fresh beef anterior lobe gave weight increases of 50% and 100%, respectively, 24 hours after administration to guinea pigs weighing between 170 and 220 gm., whose thyroid weight was normally about 25 mg. In another litter of four individuals, each of which weighed 100 gm. at the beginning of treatment, a dose of extract the equivalent of 330 mg. of fresh pars anterior was administered every other day for 8, 14, 22 and 40 days with autopsy on the day after the last injection and the weight increase in each case compared with that of controls of similar age was around 100 %. But, the total dosages given were 1320, 2310, 3630, and 6600 mg. of fresh anterior lobe. Similarly, Loeb and Friedman ('31) using guinea pigs weighing 190 to 192 gm., with control thyroid weights of 23.6 and 29.2 mg. for two groups of eight and twelve animals, secured an increase of 103% in the average thyroid weight of twelve animals given daily injections of 1 cc. of beef anterior lobe extract for 5 days and examined on the sixth and 101% increase in twelve others injected similarly for 6 days and autopsied on the seventh. But it is not known in this experiment what the total dosages of 5 and 6 cc. represent in milligrams of fresh anterior lobe. In the work of Rowlands and Parkes, doubling the weight of the 30 mg. thyroid of 200 gm. female guinea pigs serves as the basis for the 'guinea pig unit' of ox pituitary which is defined as "the thyrotropic activity contained in an amount of extract, which, given daily for 5 days, will cause the thyroids of the immature guinea-pig to attain a weight of 60 mg." ('34, p. 1838). In their tests one 'guinea pig unit' was found in 2.25 to 3.0 mg of extract. Since "the weight of the extracts obtained represented about . . . . 2% of the fresh tissue" ('36, p. 115), 112.5 to 150.0 mg. of fresh pars anterior would contain one 'guinea pig unit.' This amount must be given each day for 5 days to induce doubling of the thyroid weight by autopsy on the sixth day, so the total dose administered would be between 562.5 and 750.0 mg. of fresh pars anterior.

According to these data, a single dose of 330 mg. of fresh beef pars anterior with autopsy 24 hours later (Aron, '30 b), an average of 19.3 mg. of fresh frog pars anterior (sixteen glands) given in 4 days with autopsy on the fifth (the present experiment), 562.5 to 750.0 mg. of fresh ox pars anterior (Rowlands and Parkes, '34, '36) or 5 to 6 cc. of beef anterior lobe extract (Loeb and Friedman, '31) administered during 5 or 6 days will double the weight of the guinea pig thyroid. Or extract, the equivalent of 1320, 2310, 3630 or 6600 mg. of fresh beef anterior lobe, given in 8, 14, 22 or 40 days will do the same.[4] Obviously the dosage of frog pars anterior is extremely small compared with the mammalian ones. On the basis of Aron's statement concerning the greater sensitivity of the younger guinea pig thyroid to anterior lobe extract, it is probable that the figure for the frog dosage should be increased somewhat for older animals. To make it equivalent even to the lowest mammalian one cited above (330 mg.), it should be raised sixteen times. But it is unlikely that the amount necessary for stimulating the thyroid of a 200 gm. guinea pig would need to be so much greater than the quantity that more than doubled the thyroid weight in animals weighing about 120 gm.[5] However, this must be checked.

Furthermore, if a comparison of mammalian and anuran thyrotropic hormone is attempted on the basis of the histological picture a striking similarity in the appearance of the glands is encountered even with very different dosages. The description and photographs of the thyroid glands of guinea pigs receiving an extract containing the equivalent of 1 gm. of fresh beef anterior lobe in 1 week (Aron, '30 b, fig. 5) or of 330 mg. every other day for 8 days (Aron, '30 b, fig. 12) or "4 mg. of thyrotropic extract (ox) for 5 days" (Rowlands and Parkes, '36, fig. 6) resemble the thyroid condition in the present experiments. Also relatively few mitoses were found in the guinea pig thyroids examined on the fifth day after frog pituitary administration but this was not surprising since the height of mitotic activity according to Loeb and Bassett ('30) and Aron ('30 a) occurs on the second and third days of treatment. Moreover, the fact that the number of cells per follicle was increased over that in the controls argues for an already accomplished mitotic proliferation.

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[4] Loeb and Friedman ('33) give 1115 mg. as an average weight for anterior lobes of 3-year-old cattle. On the basis of this figure, Aron's smallest dosage (330 mg.) would be approximately one-third of a gland, that of Rowlands and Parkes one-half to two-thirds of a gland and Aron's larger dosages from one to six glands. Schockaert ('30) reported a positive reaction with one-tenth and one-half a gland, but he does not specify the exact amount of weight increase obtained. Loeb and Friedman ('33) also state that sheep, hog and cattle pituitaries in equal amounts exert about the same effect on the thyroid of the guinea pig, although possibly hog is a little more potent than sheep pars anterior. Since the sheep pars anterior weighs 350 mg. and that of the hog 125 mg. (Loeb and Friedman, '33), Aron's 330 mg. dose of cattle pars anterior would be the equivalent of one sheep gland and approximately three hog glands.

[5] Friedman and Loeb ('34) give 20.2 mg. as the average weight of the thyroid gland of six guinea pigs weighing 100 gm. Using this figure, the weight increase obtained in the present experiment would be 50%.

The data of the present experiment as well as of previous ones (Adams and Granger, '38; Adams and Tukey, '38) in which pars anterior of frogs stimulated thyroids, adrenals and reproductive tracts of immature female mice supply additional evidence against the concept of 'zoological specificity' in the hormones of the vertebrate endocrine glands.

## SUMMARY

Thyroid glands of guinea pigs about 3 weeks old at autopsy increased in weight and their follicular epithelium was stimulated by injections of eight or sixteen fresh anterior lobes of pituitaries of female Rana pipiens. After injections of frog brain tissue the thyroids of controls remained inactive. These data present additional evidence against 'zoological specificity' of the hormones of the anterior pituitary.

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# PLATE

## PLATE 1

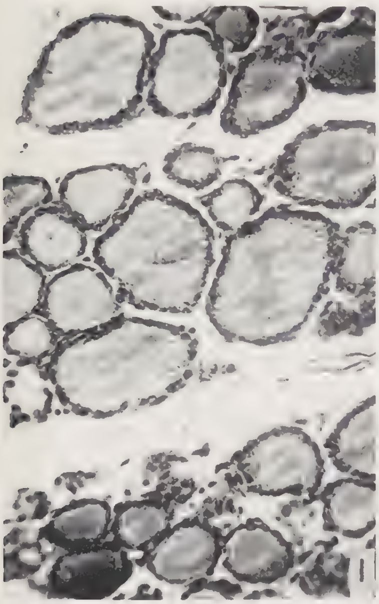
### EXPLANATION OF FIGURES

1 Thyroid of guinea pig (no. 8) that received 13.7 mg. of fresh frog brain tissue over a period of 4 days. Animal autopsied on the fifth day. × 215. Note the 'resting' condition of the follicles, especially the flattened epithelium.

2 Small part of thyroid of figure 1. × 950.

3 Thyroid of guinea pig (no. 4) that received 17.4 mg. of fresh frog pars anterior (16 P. A.) over a period of 4 days. Animal autopsied on the fifth day. × 215. Note the stimulated appearance of the thyroid, especially evident in the heightening of the follicular epithelium, increased numbers of marginal vacuoles in the colloid, decreased amount of colloid. Compare figure 1.

4 Small part of thyroid of figure 3. × 950. Compare figure 2.



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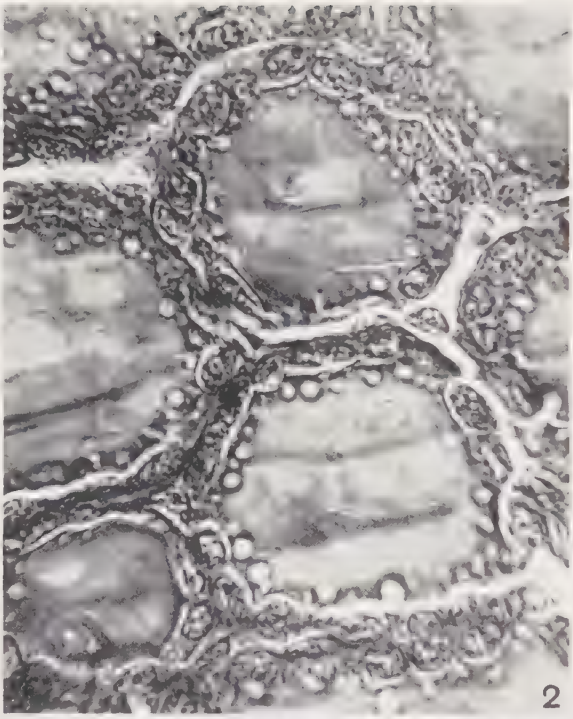

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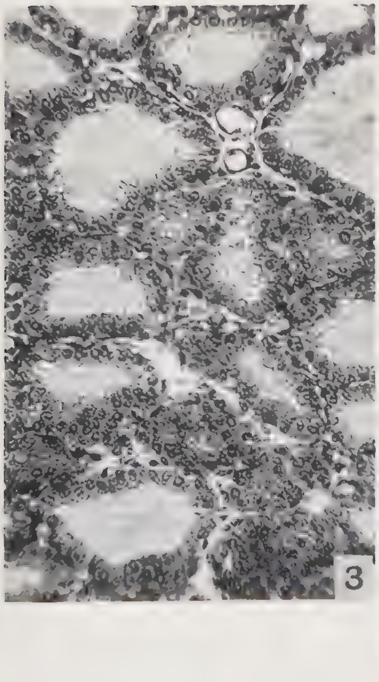

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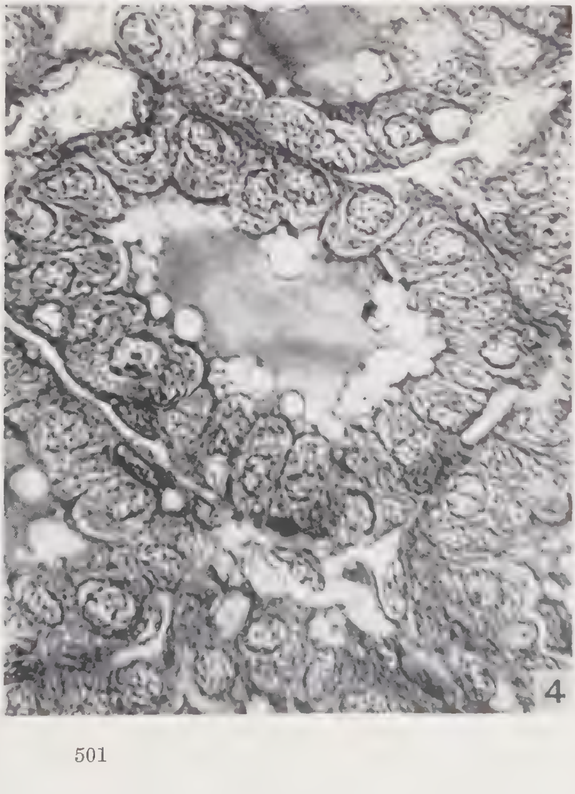

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# SUPPLEMENT

## AMERICAN SOCIETY OF ZOOLOGISTS

*Officers for 1938*

*President*........................................M. H. Jacobs
*Vice-President*...................................T. S. Painter
*Secretary*........................................E. G. Butler
*Treasurer*........................................Walter N. Hess

*Executive Committee*

(In addition to the four officers)

Charles Zeleny..........................to serve until December, 1938
A. H. Sturtevant........................to serve until December, 1939
Robert W. Hegner........................to serve until December, 1940
W. C. Allee.............................to serve until December, 1941
F. L. Hisaw.............................to serve until December, 1942

*Representative of the Society in the Division of Biology and Agriculture of the National Research Council*

To serve until July 1, 1941..............................Laurence Irving
A. C. Redfield, Alternate

*Representatives of the Society on the Council of the American Association for the Advancement of Science*

H. V. Neal    A. A. Schaeffer

*Representatives of the Society on the Council of the Union of American Biological Societies*

A. F. Shull    Sewall Wright

*Representatives of the Society on the Advisory Editorial Board of "Biological Abstracts"*

H. S. Pratt and G. K. Noble.............................*Taxonomy*
D. H. Tennent...........................................*General Embryology*
L. V. Heilbrunn.........................................*General Physiology*

*Representative on the Executive Committee of the Commission for the Standardization of Biological Stains*

S. I. Kornhauser

*Managing Editor of the Journal of Morphology*

To serve until December, 1941............................C. E. McClung

*Members of Editorial Board of the Journal of Morphology*

To serve until December, 1938
Sally Hughes-Schrader    E. E. Just    D. H. Wenrich
To serve until December, 1939
Leigh Hoadley    W. A. Kepner    J. Percy Moore
To serve until December, 1940
J. H. Bodine    N. E. McIndoo    H. H. Plough

# AMERICAN SOCIETY OF ZOOLOGISTS

## CONSTITUTION

### ARTICLE I

### NAME AND OBJECT

*Section 1.* The Society shall be called the "American Society of Zoologists."

*Sec. 2.* The object of the Society shall be the association of workers in the field of zoology for the presentation and discussion of new or important facts and problems in that science and for the adoption of such measures as shall tend to the advancement of zoological science.

### ARTICLE II

### MEMBERSHIP

*Section 1.* The membership of the Society shall consist of two classes:

*a.* Associate membership open to all actively engaged in the field of zoology who have had training in zoology equivalent to that required for the doctorate degree.

*b.* Active membership open to those who hold the doctor's degree in zoology or who have done work equivalent thereto, and who, in addition to the usual requirements for this degree, have published the results of substantial zoölogical research.

*Sec. 2.* Election to membership in the Society shall be upon recommendation of the Executive Committee.

*Sec. 3.* Associate members shall not hold office or vote, but may present papers before the Society without introduction. Associate members shall receive all literature issued by the Society, including the proceedings of the Society, and shall have the same privileges as active members in journal subscriptions.

*Sec. 4.* Associate members may be elected to active membership in the usual way when the research requirements of active membership have been met.

### ARTICLE III

### OFFICERS

*Section 1.* The officers of the Society shall be a President, a Vice-President, a Secretary, a Treasurer, and the members at large of the Executive Committee.

*Sec. 2.* The Executive Committee shall consist of the President, the Vice-President, the Secretary, the Treasurer, and five members elected from the Society at large. Of these five members, one shall be elected each year to serve five years. If any member at large shall be elected to any other office, a member at large shall be elected at once to serve the remainder of his term.

*Sec. 3.* These officers shall be elected by ballot at the annual meeting of the Society and their official terms shall commence with the close of the annual meeting, except that the Secretary and the Treasurer shall be elected triennially and shall serve for three years.

*Sec. 4.* The officers named in Section 1 shall discharge the duties usually assigned to their respective offices. (See by-laws.)

*Sec. 5.* Vacancies in the board of officers, occurring from any cause, may be filled by election by ballot at any meeting of the Society. A vacancy in either the Secretaryship or the Treasurership occurring in the interval of the meetings of the Society may be filled, until the next annual meeting, by appointment by the Executive Committee.

*Sec. 6.* At the annual meeting the President shall name a nominating committee consisting of three active members. It shall be the duty of this committee to nominate officers of the Society, including the permanent representatives which the Society is asked to name to various boards and councils. This committee shall make its nominations to the Secretary at least four months before the next annual meeting. Additional nominations for any office may be made in writing to the Secretary by any five members at any time previous to balloting.

## Article IV

### MEETINGS OF THE SOCIETY

*Section 1.* Unless previously determined by the Society, the time and place of the annual meeting shall be determined by the Executive Committee. Special meetings may be called and arranged for by the Executive Committee. Notices of such meetings shall be mailed to all members of the Society at least two weeks before the date set for the meeting.

*Sec. 2.* Sections of the Society may be organized in any locality by not less than ten members, for the purpose of holding meetings for the presentation of scientific papers. Such sections shall have the right to elect their own officers and also associate members; provided, however, that associate membership in any section shall not confer membership in the Society.

## Article V

### QUORUM

Twenty-five members shall constitute a quorum of the Society and four a quorum of the Executive Committee.

## Article VI

### CHANGES IN THE CONSTITUTION

Amendments to this Constitution may be adopted at any meeting of the Society upon approval of two-thirds of the members voting, subject to the following conditions:

*a.* The proposed amendment must be in writing and signed by at least five members of the Society.

*b*. This signed proposal must be in the hands of the Secretary at least one month before the meeting of the Society at which it is to be considered.

*c*. The Secretary shall mail copies of the proposed amendment to the members of the Society at least two weeks before the meeting.

*d*. Members unable to attend may submit their votes in writing to the Secretary.

## BY-LAWS

### ARTICLE I

### DUES

*Section 1.* The annual dues for active and associate members, unless remitted or changed by the vote of the Society, shall be two dollars.

*Sec. 2.* Any member in arrears of dues for two years should be dropped by the Executive Committee. Upon payment of arrearage he shall be reinstated.

### ARTICLE II

### PRESIDENT

*Section 1.* The President shall preside at scientific sessions and at the business meetings.

*Sec. 2.* He shall also appoint the committees prescribed by the constitution and by-laws.

### ARTICLE III

### VICE-PRESIDENT

*Section 1.* The Vice-President shall preside at sessions designated by the President.

*Sec. 2.* He shall also assume the duties of the President in the latter's absence.

### ARTICLE IV

### SECRETARY

*Section 1.* The Secretary shall keep the records of the Society.

*Sec. 2.* He shall be responsible for all arrangements for the meetings, including the appointment of local committees.

*Sec. 3.* Whenever the proper officers of a number of related societies shall have a conference covering matters of mutual interest to the several societies, he shall act as the delegate or representative of this Society.

*Sec. 4.* He shall make such purchases and employ such assistance as will, in his judgment, expedite the business of the Society, and

*Sec. 5.* He shall be reimbursed out of the funds of the Society for expenses incurred in attending meetings of the Society.

## ARTICLE V

### TREASURER

*Section 1.* The Treasurer shall be in charge of the funds of the Society.

*Sec. 2.* At the annual business meeting of the Society he shall present a statement to date of the funds of the Society.

*Sec. 3.* He shall make such purchases and employ such assistance as will, in his judgment, expedite the business of the Society.

## ARTICLE VI

### AUDITING COMMITTEE

The President shall annually appoint an auditing committee of two, who shall audit and report upon the financial record and statement of the Treasurer at the meeting for which they were appointed.

## ARTICLE VII

### PROGRAM RULES

In matters relating to programs for annual meetings, the following rules shall be observed:

*Section 1.* Titles and abstracts of papers to be presented by any method at the annual meeting must be sent to the Secretary in duplicate before the final date set by him. The length, form, and arrangement of the abstracts must comply with the requests of the Secretary in order to insure prompt publication by The Wistar Institute. The titles and abstracts of papers which do not conform to such requests or papers which are received late may be presented by title only.

*Sec. 2.* Titles shall be listed in groups according to subject matter, the groups to be determined by the Secretary.

*Sec. 3.* Whenever conditions require, the Secretary shall schedule two or more groups for the same hour and arrange the program to bring together papers on subjects of more general interest for meetings of the whole Society.

*Sec. 4.* Each member may have not more than fifteen minutes of the time of the Society at the annual meeting. This time may be used by him to present one or more papers personally or to introduce papers of non-members.

*Sec. 5.* All papers not read when called for as listed shall be placed at the end of the group list, and if not read when called for the second time, shall be read by title only.

*Sec. 6.* The titles of 'introduced' papers may be listed in the groups after the titles of papers to be read by members.

## LIST OF FORMER OFFICERS

### AMERICAN MORPHOLOGICAL SOCIETY

| *President* | *Vice-President* | *Secretary-Treasurer* |
|---|---|---|
| 1890—E. B. Wilson | ............ | J. P. McMurrich |
| 1891—C. O. Whitman | E. L. Mark | J. P. McMurrich |
| 1892—C. O. Whitman | H. F. Osborn | J. P. McMurrich |
| 1893—C. O. Whitman | E. B. Wilson | J. P. McMurrich |
| 1894—C. O. Whitman | W. B. Scott | G. H. Parker |
| 1895—E. B. Wilson | W. B. Scott | G. H. Parker |
| 1896—E. L. Mark | H. F. Osborn | G. H. Parker |
| 1897—C. S. Minot | S. I. Smith | G. H. Parker |
| 1898—H. F. Osborn | T. H. Morgan | G. H. Parker |
| 1899—E. G. Conklin | W. M. Wheeler | Bashford Dean |
| 1900—T. H. Morgan | H. C. Bumpus | J. S. Kingsley |
| 1901—J. S. Kingsley | E. A. Andrews | T. H. Montgomery |
| 1902—H. C. Bumpus | G. H. Parker | M. M. Metcalf |

### *Additional Members of the Executive Committee*

1891—E. B. Wilson
H. F. Osborn
1892—E. L. Mark
T. H. Morgan
1893—T. H. Morgan
C. B. Davenport
1894—E. A. Andrews
F. H. Herrick
1895—T. H. Morgan
S. Watase
1896—E. G. Conklin
William Patten
1897—J. S. Kingsley
Bashford Dean
1898—C. B. Davenport
F. R. Lillie
1899—J. P. McMurrich
G. H. Parker
1900—F. R. Lillie
Jacob Reighard
1901—C. F. W. McClure
C. W. Hargitt
1902—H. S. Jennings
R. G. Harrison

### AMERICAN SOCIETY OF ZOOLOGISTS

| EASTERN BRANCH | *President* | CENTRAL BRANCH |
|---|---|---|
| G. H. Parker | 1903 | Jacob Reighard |
| E. A. Andrews | 1904 | C. H. Eigenmann |
| W. E. Castle | 1905 | F. R. Lillie |
| W. E. Castle | 1906 | C. C. Nutting |
| C. B. Davenport | 1907 | S. A. Forbes |
| W. M. Wheeler | 1908 | E. A. Birge |
| H. S. Jennings | 1909 | E. A. Birge |
| T. H. Montgomery | 1910 | C. E. McClung |
| H. V. Wilson | 1911 | George Lefevre |
| A. G. Mayer | 1912 | H. B. Ward |
| Raymond Pearl | 1913 | H. B. Ward |

## LIST OF FORMER OFFICERS

| EASTERN BRANCH | *Vice-President* | CENTRAL BRANCH |
|---|---|---|
| Jacob Reighard | 1903 | H. F. Nachtrieb |
| W. E. Castle | 1904 | S. J. Holmes |
| William Patten | 1905 | William A. Locy |
| William Patten | 1906 | George Lefevre |
| F. H. Herrick | 1907 | H. B. Ward |
| H. S. Jennings | 1908 | M. F. Guyer |
| H. V. Wilson | 1909 | M. F. Guyer |
| H. H. Wilder | 1910 | H. F. Nachtrieb |
| H. E. Crampton | 1911 | R. H. Walcott |
| G. A. Drew | 1912 | C. M. Child |
| Alex. Petrunkevitch | 1913 | C. M. Child |

| | *Secretary-Treasurer* | |
|---|---|---|
| G. A. Drew | 1903 | Frank Smith |
| G. A. Drew | 1904 | F. R. Lillie |
| H. S. Pratt | 1905 | C. E. McClung |
| H. S. Pratt | 1906 | T. G. Lee |
| C. J. Herrick | 1907 | T. G. Lee |
| L. L. Woodruff | 1908 | T. G. Lee |
| L. L. Woodruff | 1909 | Charles Zeleny |
| H. W. Rand | 1910 | H. V. Neal |
| Raymond Pearl | 1911 | H. V. Neal |
| J. H. Gerould | 1912 | W. C. Curtis |
| Caswell Grave | 1913 | W. C. Curtis |

*Executive Committeemen*

| | |
|---|---|
| F. R. Lillie | Herbert Osborn |
| T. H. Montgomery | C. H. Eigenmann |
| H. C. Bumpus | J. G. Needham |
| H. S. Jennings | S. J. Holmes |
| E. A. Andrews | W. A. Locy |
| W. R. Coe | C. M. Child |
| G. A. Drew | R. H. Walcott |
| M. M. Metcalf | W. C. Curtis |
| D. H. Tennent | Oscar Riddle |
| R. G. Harrison | H. B. Ward |
| H. E. Jordan | Chauncey Juday |
| C. E. McClung | H. W. Norris |
| George Lefevre | C. E. McClung |
| T. G. Lee | H. F. Nachtrieb |

## American Society of Zoologists (Amalgamated)

| | *President* | *Vice-President* | *Executive Committeemen* |
|---|---|---|---|
| 1914. | C. E. McClung | M. F. Guyer | H. E. Jordan—1 year |
| 1915. | W. A. Locy | W. E. Ritter | H. F. Nachtrieb—2 years |
| 1916. | D. H. Tennent | Charles Zeleny | H. V. Wilson—3 years |
| 1917. | M. M. Metcalf | Charles Zeleny | George Lefevre—4 years |
| 1918. | George Lefevre | L. L. Woodruff | A. F. Shull—5 years |
| 1919. | C. M. Child | H. H. Wilder | D. H. Tennent—5 years |
| 1920. | Gilman A. Drew | Caswell Grave | R. P. Bigelow—5 years |
| 1921. | Charles A. Kofoid | Aaron T. Treadwell | L. J. Cole—4 years |
| 1922. | H. H. Wilder | Bennet M. Allen | H. V. Wilson—5 years |
| 1923. | M. F. Guyer | Robert A. Budington | M. M. Metcalf—5 years |
| 1924. | R. G. Harrison | R. K. Nabours | George Lefevre—5 years |
| 1925. | C. R. Stockard | C. R. Moore | C. M. Child—5 years |
| 1926. | S. O. Mast | W. C. Allee | G. A. Drew—5 years |
| 1927. | S. J. Holmes | Robert Chambers, Jr. | C. A. Kofoid—5 years |
| 1928. | Caswell Grave | M. H. Jacobs | H. H. Wilder—5 years |
| 1929. | C. B. Davenport | Fernandus Payne | J. H. Gerould—1 year |
| 1930. | H. V. Neal | E. E. Just | M. F. Guyer—5 years |
| 1931. | Fernandus Payne | D. E. Minnich | R. G. Harrison—5 years |
| 1932. | W. C. Curtis | L. V. Heilbrunn | C. R. Stockard—5 years |
| 1933. | Charles Zeleny | J. H. Bodine | S. O. Mast—5 years |
| 1934. | A. H. Sturtevant | H. W. Rand | S. J. Holmes—5 years |
| 1935. | R. W. Hegner | Sewall Wright | Caswell Grave—5 years |
| 1936. | W. C. Allee | C. W. Metz | C. B. Davenport—4 years |
| 1937. | F. L. Hisaw | Helen D. King | H. V. Neal—5 years |
| | | | Fernandus Payne—5 years |
| | | | W. C. Curtis—5 years |

*Secretary-Treasurer*

1914–1918. Caswell Grave
1918–1921. W. C. Allee

| | *Secretary* | | *Treasurer* |
|---|---|---|---|
| 1921–1924. | W. C. Allee | 1922. | D. H. Tennent |
| 1925–1928. | D. E. Minnich | 1923–1924. | R. H. Bowen |
| 1928–1929. | L. B. Arey | 1925–1930. | L. B. Arey |
| 1929–1930. | D. E. Minnich | 1930–1933. | A. B. Dawson |
| 1930–1933. | W. H. Cole | 1933–1935. | B. H. Willier |
| 1933–1936. | H. B. Goodrich | | |

*Representative in the Division of Biology and Agriculture, National Research Council*

M. F. Guyer 1919–1921
William Patten 1921–1924
J. T. Patterson 1924–1927
G. H. Parker 1926–1929
G. H. Parker 1919–1922
H. S. Jennings 1922–1925
L. L. Woodruff 1925–1928
E. G. Conklin 1926–1929
F. R. Lillie 1919–1923
E. G. Conklin 1923–1926
H. S. Jennings 1929–1931
L. C. Dunn 1931–1932
J. H. Bodine 1932–1935
L. V. Heilbrunn 1935–1938

### *Associate Editors of the Journal of Morphology*

| | | | |
|---|---|---|---|
| 1921. | Gary N. Calkins | J. S. Kingsley | William Patten |
| 1921–1922. | E. G. Conklin | M. F. Guyer | W. M. Wheeler |
| 1921–1923. | C. A. Kofoid | F. R. Lillie | J. T. Patterson |
| 1922–1924. | G. A. Drew | H. V. Neal | L. L. Woodruff |
| 1923–1925. | Caswell Grave | D. H. Tennent | H. V. Wilson |
| 1924–1926. | Helen Dean King | S. O. Mast | A. A. Schaeffer |
| 1925–1927. | R. W. Hegner | Fernandus Payne | H. B. Ward |
| 1926–1928. | L. J. Cole | J. H. Gerould | M. H. Jacobs |
| 1927–1929. | W. C. Curtis | J. Percy Moore | A. F. Shull |
| 1928–1930. | Edgar Allen | G. A. Baitsell | R. E. Coker |
| 1929–1931. | S. R. Detwiler | R. S. Lillie | H. J. Muller |
| 1930–1932. | W. R. Coe | E. N. Harvey | H. E. Jordan |
| 1931–1933. | L. V. Heilbrunn | S. O. Mast | G. K. Noble |
| 1932–1934. | D. E. Minnich | B. H. Willier | L. L. Woodruff |
| 1933–1935. | L. R. Cleveland | F. L. Hisaw | Franz Schrader |
| 1934–1936. | Charles Zeleny | J. F. Daniel | J. S. Nicholas |
| 1935–1937. | D. H. Tennent | L. C. Dunn | C. G. Hartman |

## LIST OF PLACES OF MEETING

### American Morphological Society

1890—Boston
1891—Philadelphia
1892—Princeton
1893—New Haven
1894—Baltimore
1895—Philadelphia
1896—Boston
1897—Ithaca
1898—New York
1899—New Haven
1900—Baltimore
1901—Chicago
1902—Washington

### Central Naturalists

1899—Chicago
1900—Chicago

### Society of American Zoölogists

1901—Chicago
1902—Washington

### American Society of Zoologists

#### EASTERN BRANCH

1903—Philadelphia
1904—Philadelphia
1906—New York
1907—New Haven
1909—Boston
1910—Ithaca

#### JOINT MEETINGS

1905—Ann Arbor
1908—Baltimore
1911—Princeton
1912—Cleveland
1913—Philadelphia

#### CENTRAL BRANCH

1903—St. Louis
1905—(Mch.) Chicago
1907—(Mch.) Madison
1907—Chicago
1910—(Apr.) Iowa City
1910—Minneapolis
1912—(Apr.) Urbana

#### MEETING PLACES

1914—Philadelphia
1915—Columbus
1916—New York
1917—Minneapolis
1918—Baltimore
1919—St. Louis
1920—Chicago
1921—Toronto
1922—Boston
1923—Cincinnati
1924—Washington
1925—New Haven
1926—Philadelphia
1927—Nashville
1928—New York
1929—Des Moines
1930—Cleveland
1931—New Orleans
1932—Atlantic City
1933—Boston
1934—Pittsburgh
1935—Princeton
1936—Atlantic City
1937—Indianapolis

# PROGRAM FOR THE THIRTY-SIXTH ANNUAL MEETING OF THE AMERICAN SOCIETY OF ZOOLOGISTS

RICHMOND, VIRGINIA

DECEMBER 28, 29, 30, 1938

The Society will meet this year in conjunction with Section F of the American Association for the Advancement of Science and in association with other biological societies. All meetings will be held at the Medical College of Virginia, the Academy of Medicine or nearby buildings.

Several special sessions have been arranged. A symposium on 'Chromosome Structure,' organized by Dr. Theophilus S. Painter, will be held jointly with the Genetics Society of America. Dr. G. H. Parker will lead a symposium on 'Color Changes in Animals, their Significance and Activation.' Dr. H. S. Jennings is in charge of a symposium on 'Mating Types and their Interactions in Ciliate Infusoria; Determination, Inheritance and Relation to Sexuality.' In addition, the Society will join with the American Society of Naturalists in sponsoring a symposium on 'Contributions of the Higher Animals to an Understanding of Human Biology.'

The attention of members of the Society is directed also to the symposium to commemorate the Centenary of the Cell Theory, which will be held on Tuesday, December 27th (the day preceding the opening meeting of the American Society of Zoologists). This symposium has been arranged through collaboration of Sections F, G and L of the American Association for the Advancement of Science.

The Biologists' Smoker will be held on the first evening of the meeting, Wednesday, December 28th, at 9.15 o'clock at the John Marshall Hotel.

The Annual Dinner of the American Society of Zoologists, to which all zoologists and friends are invited, will be held at

7.00 o'clock Thursday evening, December 29th, at the Hotel Richmond. Dr. F. B. Sumner, vice-president of Section F of the A.A.A.S. will give an address on 'Human Psychology and Some Things that Fishes Do.'

The Secretary has received no notification of special reduction in railroad fares for the Richmond meeting. However, members are advised to consult their local railroad ticket agents in regard to possible convention or holiday rates.

As in former years the Society is greatly indebted to The Wistar Institute for prompt publication and mailing of this program.

## LOCATION OF MEETINGS

The *Academy of Medicine,* in which all sessions of the Society will be held on Wednesday, Thursday and Friday forenoons, is situated at the corner of 12th and Clay Streets. The demonstration program on Thursday afternoon will be held in the laboratories of *McGuire Hall* (*Medical College of Virginia*), directly across the street from the Academy of Medicine.

The *John Marshall High School,* where the joint symposium with the Genetics Society will be held on Wednesday afternoon, is at 9th and Marshall Streets, four blocks from the Academy of Medicine and two blocks from the Hotel Richmond.

The *Hotel Richmond,* which will serve as hotel headquarters for the Society, is located at 9th and Grace Streets, facing Capitol Square. This hotel is six short blocks from the Academy of Medicine and McGuire Hall.

## LUNCHEON AND DINNER TICKETS

Arrangements have been made for Biologists' Luncheons on Wednesday, Thursday and Friday in the Men's Dormitory of the Medical College, one block from the Academy of Medicine. Luncheon tickets can be secured at the information desk in the Academy of Medicine.

Tickets for the Zoologists' Dinner are also on sale at the information desk. In order to assist the Local Committee in making arrangements, members of the Society are urged to obtain dinner tickets as early as possible.

# PROGRAM

Sections F, G and L of the American Association for the Advancement of Science are collaborating in a symposium to commemorate the *Centenary of the Cell Theory.* This symposium will be held on both morning and afternoon, Tuesday, December 27th, at the Jefferson Hotel. Among the speakers will be E. G. Conklin, L. L. Woodruff, J. S. Karling, Franz Schrader and Paul Weiss. For further details regarding this symposium, consult the General Program of the A.A.A.S.

## WEDNESDAY, DECEMBER 28TH

9.30 A.M. Section A *Experimental Embryology.* Papers 1 to 10. Room 20, Academy of Medicine.

Section B. *Endocrinology.* Papers 11 to 22. Auditorium, Academy of Medicine.

Section C. *General Physiology.* Papers 23 to 38. Room 10, Academy of Medicine.

Section D. *Protozoology and Parasitology.* Papers 39 to 49. Room 30, Academy of Medicine

11.30 to 2.00 *Biologists' Luncheon.* Men's Dormitory (12th and Marshall Streets).

2.00 P.M. Symposium on *Chromosome Structure.* Joint Session with the Genetics Society of America. Auditorium, John Marshall High School (9th and Marshall Streets). Theophilus S. Painter, presiding.

1. The structure of large plant chromosomes. B. R. Nebel, New York State Agricultural Experiment Station, Geneva.
2. The structure of the salivary gland chromosomes. T. S. Painter, University of Texas.
3. The physico-chemical nature of the chromosome and the gene. C. H. Waddington, University of Cambridge.
4. Chromosome structure as viewed by the geneticist. M. Demerec, Carnegie Institution of Washington.

9.15 P.M. *Biologists' Smoker.* John Marshall Hotel (5th and Franklin Streets).

## THURSDAY, DECEMBER 29TH

9.00 A.M. Section A. Symposium on *Color Changes in Animals, their Significance and Activation.* G. H. Parker, presiding. (Program on page 19.) Auditorium, Academy of Medicine.

Section B. *Endocrinology.* Papers 50 to 63. Room 20, Academy of Medicine.

Section C. *Cellular Physiology.* Papers 64 to 75. Room 10, Academy of Medicine.

Section D. *Experimental Embryology.* Papers 76 to 85. Room 30, Academy of Medicine.

12.00 M. Annual Business Meeting of Section F, A.A.A.S., Auditorium, Academy of Medicine.

12.10 P.M. Annual Business Meeting of the American Society of Zoologists, Auditorium, Academy of Medicine.

11.30 to 2.00 *Biologists' Luncheon.* Men's Dormitory (12th and Marshall Streets).

2.00 P.M. *Demonstration Program.* Papers 120 to 142A. Third floor, McGuire Hall, Medical College of Virginia. (Motion pictures in Room 20, Academy of Medicine.) See page 22.

7.00 P.M. *Zoologists' Dinner.* Address by Dr. F. B. Sumner, vice-president of Section F of the A.A.A.S. on *Human Psychology and Some Things that Fishes Do.* Winter Garden, Hotel Richmond. Open to all zoologists and friends.

### FRIDAY, DECEMBER 30TH

9.30 A.M. Section A. Symposium on *Mating Types in Ciliate Infusoria; Determination, Inheritance, and Relation to Sexuality; with Demonstrations.* H. S. Jennings, presiding. (Program on page 23.) Auditorium, Academy of Medicine.

Section B. *Cytology* and *Histology.* Papers 86 to 97. Room 10, Academy of Medicine.

Section C. *General Physiology.* Papers 98 to 110. Room 20, Academy of Medicine.

Section D. *General Morphology and Miscellaneous.* Papers 111 to 119. Room 30, Academy of Medicine.

11.30 to 2.00 *Biologists' Luncheon.* Men's Dormitory (12th and Marshall Streets).

2.00 P.M. Symposium on *Contributions of the Higher Animals to an Understanding of Human Biology.* Joint Session with the American Society of Naturalists. Auditorium, John Marshall High School (9th and Marshall Streets).

1. The experimental animal from the naturalists' point of view. G. K. Noble, American Museum of Natural History.
2. Some contributions of the laboratory rodents to our understanding of human biology. C. C. Little, Roscoe B. Jackson Memorial Laboratory.
3. The use of the monkey for the study of human biology. Carl G. Hartman, Carnegie Institution of Washington, Department of Embryology.

The Naturalists' Dinner will be held on Friday evening, December 30th, 6.30 P.M. at the John Marshall Hotel. The presidential address will be given by Dr. Robert M. Yerkes, Yale University, on 'The Life History and Personality of the Chimpanzee.'

# LIST OF TITLES

## WEDNESDAY MORNING SESSION, DECEMBER 28TH

*Academy of Medicine, 12th* and *Clay Streets*

This session will be held in four sections, as shown below.

Section A. *Experimental Embryology,* 9.30 A.M.; Room 20, Academy of Medicine

1. An analysis of lithium microcephaly in Rana pipiens. (10 min.) (Lantern.) T. S. Hall, Yale University, (Introduced by J. S. Nicholas.)
2. Experiments on the time of localization of the organizing center in the egg of the Japanese newt. (15 min.) (Lantern.) J. Clark Streett, Jr., Princeton University. (Introduced by Ulric Dahlgren.)
3. The effects of extirpation of the notochord, undertaken at the medullary plate stage, in Amblystoma mexicanum. (12 min.) (Lantern.) Irwin C. Kitchin, The Rice Institute. (Introduced by Edgar Altenburg.)
4. Metabolic properties of the regions of the amphibian gastrula. (15 min.) (Lantern.) E. J. Boell, Yale University. (Introduced by G. A. Baitsell.)
5. The nature and mode of action of chemical evocators in embryonic induction in amphibia. (15 min.) (Lantern.) C. H. Waddington, University of Cambridge, England. (Introduced by L. C. Dunn.)
6. Modification of development in the sand dollar with NaCNS and Ca-free sea water. (15 min.) (Lantern.) Olin Rulon, Wayne University.
7. Development of parts of Chaetopterus eggs, with special reference to parthenogenetic merogony. (15 min.) (Lantern.) Ethel Browne Harvey, Princeton University.
8. The effect of shape on the polarity of the Fucus egg. (15 min.) (Lantern.) D. M. Whitaker, Stanford University.
9. Retardation of cell division by irradiation with ultraviolet light. (15 min.) (Lantern.) Arthur C. Giese, Stanford University. (Introduced by C. V. Taylor.)
10. A possible explanation of how x-rays may produce both death and malignant growth in cells based on Boveri's theory of cancer and certain experimental observations. (15 min.) (Lantern.) Paul S. Henshaw, Memorial Hospital, New York City, and National Cancer Institute, Washington.

The program in Embryology will be continued Thursday morning with paper no. 76.

Section B. *Endocrinology*, 9.30 A.M., Auditorium, Academy of Medicine

11. Experimental studies on the reproductive system of the male anuran. (15 min.) (Lantern.) Roberts Rugh, Hunter College.
12. An analysis of gonadal weights in adult Triturus viridescens in relation to the annual reproductive cycle. (15 min.) (Lantern.) A. Elizabeth Adams, Mount Holyoke College.
13. The pars tuberalis of the hypophysis in toads. (15 min.) (Lantern.) Wayne J. Atwell, University of Buffalo.
14. Augmentation of pituitary extract by chlorophyll plant growth hormones and hemin. (10 min.) (Lantern.) W. R. Breneman, Waterman Institute and Department of Zoology, Indiana University.
15. The morphology of the thyroid gland in the metamorphosing Rana pipiens. (10 min.) Savino A. D'Angelo and Harry A. Charipper, Washington Square College, New York University. (Introduced by Morris H. Harnly.)
16. The 'critical' period in the metamorphosis of Rana sylvatica. (10 min.) (Lantern.) Savino A. D'Angelo, Albert S. Gordon and Harry A. Charipper, Washington Square College, New York University.
17. The reticulo-endothelial system and hormone refractoriness. IV. The anti-thyrotropic complex. (15 min.) (Lantern.) Albert S. Gordon, Savino A. D'Angelo and Harry A. Charipper.
18. The production of experimental exophthalmos and the effect of thyroxin and other iodine compounds on its development. (15 min.) (Lantern.) George K. Smelser, Columbia University.
19. Effect of bilateral optic enucleation on body weights of rats under various light conditions. (15 min.) (Lantern.) Ludvig G. Browman, Montana State University.
20. On the nature and action of diantlin, a new hormone carried by the spermatozoa of the oyster. (15 min.) (Lantern.) T. C. Nelson and J. B. Allison, Bureau of Biological Research, Rutgers University.
21. Sex change and physiological intersexuality in Ostrea virginica. (15 min.) (Lantern.) Paul S. Galtsoff, U. S. Bureau of Fisheries.
22. On the role of certain hormones in organic synthesis. (10 min.) (Lantern.) F. H. Pike and R. L. Zwemer, Columbia University.

The program in Endocrinology will be continued Thursday morning with paper no. 50.

Section C. *General Physiology*, 9.30 A.M.; Room 10, Academy of Medicine

23. Sequence of appearance of x-radiation effects. (8 min.) (Lantern.) Titus C. Evans, State University of Iowa.
24. Effects of roentgen rays on the activation and production of the enzyme tyrosinase in the insect egg (Orthoptera). (7 min.) (Lantern.) O. Malcolm Ray, State University of Iowa. (Introduced by Titus Carr Evans.)
25. Ontogeny of the natural activator of the enzyme, tyrosinase. (7 min.) (Lantern.) J. H. Bodine, O. Malcolm Ray and Thomas H. Allen, State University of Iowa.
26. The effect of pyocyanin on the respiratory mechanism of the grasshopper embryo (Melanoplus differentialis). (8 min.) (Lantern.) Loren D. Carlson, State University of Iowa. (Introduced by J. H. Bodine.)

27. Respiratory quotients of the female honeybee during larval and pupal development. (12 min.) (Lantern.) R. M. Melampy and E. R. Willis, U. S. Department of Agriculture and Louisiana State University.
28. Pigment metabolism studies. (15 min.) (Lantern.) Frank H. J. Figge, Department of Anatomy, University of Maryland Medical School. (Introduced by Eduard Uhlenhuth.)
29. The affinity of hemoglobin for oxygen in marine fishes. (8 min.) (Lantern.) F. G. Hall, Duke University.
30. The role of hemoglobin in amphibian development and metamorphosis. (7 min.) (Lantern.) F. H. McCutcheon, North Carolina State College. (Introduced by F. G. Hall.)
31. The effect of $CO_2$ on the $O_2$-combining power of whole and hemolyzed marine fish blood. (15 min.) (Lantern.) R. W. Root, E. C. Black and Laurence Irving, College of the City of New York and Swarthmore College.
32. Oxygen transport by the blood of the carp and the common sucker. (15 min.) (Lantern.) E. C. Black, University of Pennsylvania and Swarthmore College. (Introduced by Laurence Irving.)
33. The influence of carbon dioxide on the utilization of oxygen by certain species of fish in Algonquin Park, Ontario. (15 min.) (Lantern.) F. E. J. Fry, Ontario Fisheries Research Laboratory and University of Toronto, and E. C. Black, University of Pennsylvania and Swarthmore College. (Introduced by Frank R. Kille.)
34. Metabolism studies of the development of temperature regulation in birds. (10 min.) (Lantern.) S. Charles Kendeigh, Baldwin Bird Research Laboratory and University of Illinois.
35. Cyclic variation in the heart rate of wild birds. (5 min.) (Lantern.) Eugene P. Odum, Baldwin Bird Research Laboratory and University of Illinois. (Introduced by S. C. Kendeigh.)
36. Chemical mediators and the heart of Panulirus argus. (10 min.) (Lantern.) John H. Welsh, Harvard University.
37. The blood picture and the blood sugar changes during the alarm reaction. (15 min.) (Lantern.) Albert J. Dalton and Hans Selye, McGill University.
38. Temperature activation of impure and crystalline urease. (15 min.) (Lantern.) Irwin W. Sizer, Massachusetts Institute of Technology.

The program in Physiology will be continued Thurday morning with paper no. 64.

### Section D. *Protozoology and Parasitology*, 9.30 A.M.; Room 30, Academy of Medicine

39. Some aspects of spiraling in ciliates, trees, and vines. (15 min.) (Lantern.) W. E. Bullington, Randolph Macon College.
40. Effect of osmotic pressure on the motility and viability of fresh-water protozoa. (10 min.) (Lantern.) John B. Loefer, Berea College.
41. Effect of the addition of old culture medium on the growth of Colpidium campylum. (8 min.) (Lantern.) R. P. Hall and J. B. Loefer, New York University and Berea College.

42. Growth of Astasia sp. and Euglena gracilis in media containing inorganic nitrogen. (12 min.) (Lantern.) Henry W. Schoenborn, New York University. (Introduced by R. P. Hall.)
43. The blocking of excystment reactions of Colpoda duodenaria by absence of oxygen. (10 min.) (Lantern.) Morden G. Brown, Stanford University. (Introduced by C. V. Taylor.)
44. Encystment in the ciliate, Woodruffia metabolica. (12 min.) (Lantern.) Willis H. Johnson, Stanford University.
45. Regeneration in the genus Paramecium. (8 min.) Vance Tartar, Yale University. (Introduced by L. L. Woodruff.)
46. Racial differences within the species Chaos diffluens Müller, the common laboratory ameba. (15 min.) (Motion picture.) (Lantern.) A. A. Schaeffer, Temple University.
47. Some products of the cellulose dissimilation by the termite protozoa. (15 min.) (Lantern.) R. E. Hungate, University of Texas. (Introduced by J. T. Patterson.)
48. Life-cycle of a sporozoan parasite of the oyster. (15 min.) (Lantern.) Herbert F. Prytherch, U. S. Bureau of Fisheries.
49. Haptophrya virginiensis nov. sp., a protozoan parasite of the pickerel frog, Rana palustris Le Conte. (7 min.) (Lantern.) Samuel Lewis Meyer. Miller School of Biology, University of Virginia. (Introduced by L. L. Woodruff.)

## WEDNESDAY AFTERNOON SESSION, DECEMBER 28TH

2.00 P.M., Auditorium, John Marshall High School, *9th* and *Marshall Streets*

*Joint Symposium with the Genetics Society of America*

### CHROMOSOME STRUCTURE

*Theophilus S. Painter, presiding*

(1) The structure of large plant chromosomes. B. R. Nebel, New York State Agricultural Experiment Station, Geneva.
(2) The structure of the salivary gland chromosomes. T. S. Painter, University of Texas.
(3) The physico-chemical nature of the chromosome and the gene. C. H. Waddington, University of Cambridge.
(4) Chromosome structure as viewed by the geneticist. M. Demerec, Carnegie Institution of Washington.

## WEDNESDAY EVENING SESSION, DECEMBER 28TH

*Biologists' Smoker,* 9.15 P.M.; John Marshall Hotel, *5th* and *Franklin Streets*

## THURSDAY MORNING SESSION, DECEMBER 29TH

*Academy of Medicine, 12th* and *Clay Streets*

This session will be held in four sections, as shown below.

### Section A. *Symposium,* 9.00 A.M.; Auditorium, Academy of Medicine

### COLOR CHANGES IN ANIMALS, THEIR SIGNIFICANCE AND ACTIVATION

*G. H. Parker, presiding*

(1) Introductory remarks. G. H. Parker, Harvard University.

(2) Chromatophores in relation to specific and genetic distinctions. H. B. Goodrich, Wesleyan University.

(3) The pituitary control of chromatophores in the dogfish. A. A. Abramowitz, Harvard University.

(4) Quantitative effects of visual stimuli upon pigmentation. F. B. Sumner, University of California.

(5) The responses of melanophores in isolated fish scales. D. C. Smith, University of Maryland, School of Medicine.

(6) Humoral control of crustacean chromatophores. F. A. Brown, Jr., Northwestern University.

### Section B. *Endocrinology,* 9.00 A.M.; Room 20, Academy of Medicine

50. The corpus luteum of reptiles. (15 min.) (Lantern.) Hermann Rahn, The Biological Laboratories of Cold Spring Harbor and Harvard University. (Introduced by A. B. Dawson.)
51. The role of estrogens and progesterone in the induction of mounting activity in the spayed female guinea pig. (15 min.) (Lantern.) William C. Young, John L. Boling and Brewster Rundlette, Brown University and Yale University.
52. A description of the 'heat response' given at birth by young male and female guinea pigs. (10 min.) John L. Boling, William C. Young, James G. Wilson and Richard J. Blandau, Brown University. (Introduced by A. D. Mead.)
53. Data bearing on the time required for parturition and on postparturitional heat in the guinea pig. (5 min.) John L. Boling, Richard J. Blandau, Arnold L. Soderwall, William R. Fish and William C. Young, Brown University. (Introduced by A. D. Mead.)
54. Cyclic changes in the follicular apparatus of the ovary of the adult rat. (15 min.) (Lantern.) Charles E. Lane, University of Wichita.
55. The changes in the vaginal smears and associated cyclic phenomena in the lowland gorilla (Gorilla gorilla). (5 min.) Charles R. Noback, Washington Square College, New York University. (Introduced by Harry A. Charipper.)
56. Role of progesterone and other sex hormones in survival of pseudopregnant, adrenalectomized ferrets. (10 min.) (Lantern.) Robert Gaunt and H. W. Hays, Washington Square College, New York University and Princeton University.

57. Cortical hormone-like action of progesterone in rats. (5 min.) (Lantern.) Robert Gaunt, Warren O. Nelson and Eleanor Loomis, Biological Laboratory, Cold Spring Harbor; Department of Biology, Washington Square College, New York University, and Department of Anatomy, Wayne University.
58. Tests indicating absence of progesterone in fowl ovary. (10 min.) (Lantern.) Oscar Riddle and James P. Schooley, Carnegie Institution.
59. Effects of estrogens and estrogens plus androgens upon the testes and gonaducts of adult male pigeons. (15 min.) (Lantern.) Carroll A. Pfeiffer and W. U. Gardner, Yale University. (Introduced by Edgar Allen.)
60. Effect of testosterone propionate on the black-crowned night heron. (12 min.) (Lantern.) G. K. Noble and M. Wurm, American Museum of Natural History.
61. Influence of injected male hormone on the social hierarchy in small flocks of hens. (15 min.) (Lantern.) W. C. Allee and Nicholas Collias, The University of Chicago.
62. Effects of hypophysectomy and adrenalectomy on renal activity in the rat. (10 min.) (Lantern.) E. L. Corey, University of Virginia Medical School.
63. Intra-ocular homotransplantation of suprarenal glands in the rat. (10 min.) (Lantern.) C. Donnell Turner, Northwestern University.

Section C. *Cellular Physiology,* 9.00 A.M., Room 10, Academy of Medicine

64. The relation between the age of the culture from which Chilomonas is taken and the rate of reproduction in fresh culture fluid. (15 min.) (Lantern.) S. O. Mast and D. M. Pace, Johns Hopkins University.
65. The adjustment of the marine amoeba, Flabellula mira Schaeffer, to changes in the total salt concentration of the external medium. (15 min.) (Lantern.) Dwight L. Hopkins, Mundelein College.
66. The functions of contractile vacuoles. (15 min.) (Lantern.) J. A. Kitching, University of Bristol, England. (Introduced by E. Newton Harvey.)
67. The sperm-aster and protoplasmic movement. (15 min.) (Lantern.) Robert Chambers, New York University, Washington Square College.
68. Structure of the intra-nuclear chromosome. (15 min.) (Motion picture.) (Lantern.) William R. Duryee, New York University, Washington Square College.
69. The effect of changes of urine pH on the elimination of neutral red and phenol red by the perfused frog kidney. (15 min.) (Lantern.) Rudolf T. Kempton, Vassar College.
70. Oxygen consumption and methylene blue reduction in relation to veronal inhibition of luminescence. (10 min.) (Lantern.) Frank H. Johnson and Edward L. Chambers, Princeton University.
71. The bactericidal action of high and low intensities of ultraviolet radiation. (15 min.) (Lantern.) H. C. Rentschler and Rudolf Nagy, Research Laboratory of the Lamp Division, Westinghouse Electric and Manufacturing Co. (Introduced by W. C. Curtis.)

72. The effect of potassium and of urea on the breakdown of the pigment granules in the fertilized egg of Arbacia. (15 min.) (Motion picture.) (Lantern.) Leon Churney, University of Pennsylvania. (Introduced by L. V. Heilbrunn.)
73. The relation of the calcium ion to the irritability of isolated muscle fibers. (15 min.) (Lantern.) L. V. Heilbrunn and E. W. Ashkenaz, University of Pennsylvania.
74. The influence of temperature upon the dynamic constants of muscle. (10 min.) (Lantern.) Herbert Shapiro, University College, London, and Clark University.
75. The behavior of the cross striae of muscle fibers and the significance for certain problems of muscle organization. (12 min.) (Lantern.) Carl Caskey Speidel, University of Virginia.

The program in Physiology will be continued Friday morning with paper no. 98.

Section D. *Experimental Embryology*, 9.00 A.M.; Room 30, Academy of Medicine

76. Time-temperature relations in whitefish incubation. (12 min.) (Lantern.) John W. Price, Ohio State University.
77. The production of robin pigment in white leghorn feather cells by skin ectoderm grafts of the robin embryo. (15 min.) (Lantern.) Mary E. Rawles, University of Rochester.
78. Mosaic development and regeneration of the eye-forming area in the chick embryo. (15 min.) (Lantern.) Nelson T. Spratt, Jr., University of Rochester. (Introduced by B. H. Willier.)
79. The effect of homotypically conditioned water and the size of the container on the growth of Amblystoma larvae. (10 min.) (Lantern.) Cranford Hutchinson, The Morris Biological Farm of The Wistar Institute. (Introduced by Helen Dean King.)
80. Further experiments upon axial polarization in the embryonic limb rudiment of the salamander. (10 min.) (Lantern.) F. H. Swett, Duke University School of Medicine.
81. Sex differentiation in triploid salamanders (Triturus viridescens). (15 min.) (Lantern.) Gerhard Fankhauser, Princeton University.
82. Androgenetic development of the frog egg, Rana pipiens. (15 min.) (Lantern.) Keith R. Porter, Harvard University and Princeton University. (Introduced by Leigh Hoadley.)
83. An analysis of testis and duct shape in Drosophila. (15 min.) (Lantern.) Curt Stern, University of Rochester.
84. Chromosomal deficiencies and embryonic determination in eggs of Drosophila melanogaster. (10 min.) (Lantern.) D. F. Poulson, Yale University.
85. Cytoplasmic influence in inheritance in Daphnia. (15 min.) (Lantern.) A. M. Banta and Thelma R. Wood, Brown University.

## BUSINESS MEETING

The *Annual Business Meeting of Section F., A. A. A. S.* will be held on Thursday, December 29th, 12 M.; Auditorium, Academy of Medicine. The *Annual Business Meeting of the American Society of Zoologists* will be held in the same room at 12.10 P.M., immediately after the meeting of Section F.

## THURSDAY AFTERNOON SESSION, DECEMBER 29TH

### Demonstration Program

Beginning at 2.00 P.M.; *General Demonstrations,* Third floor, McGuire Hall, Medical College of Virginia. (Motion pictures, Room 20, Academy of Medicine)

*Motion picture demonstrations*

120. Cinéphotomicrographs of skeletal and cardiac muscle fibers in action. (15 min.) Carl Caskey Speidel, University of Virginia. Motion picture to be shown at 3.00 P.M.; Room 20, Academy of Medicine.
121. A new type of protoplasmic movement. (15 min.) W. J. Baumgartner, University of Kansas. Motion picture to be shown at 3.15 P.M.; Room 20, Academy of Medicine.

*Cytology*

122. Effects of ultracentrifuging and colchicine on various types of cells. H. W. Beams and R. L. King, State University of Iowa.
123. Visible organization of the salivary gland chromosomes of Diptera. C. W. Metz, Carnegie Institution of Washington.
124. Alteration of mitochondrial pattern in the hepatic cells of the chick embryo. Albert J. Dalton, Western Reserve University and McGill University.
125. Demonstration of the chromosomes of the chick. Robert T. Hance, University of Pittsburgh.
126. Demonstrations of new histological methods. Robert T. Hance, University of Pittsburgh.
127. Morphogenesis of transplanted hind limbs of chick embryos. Herbert L. Eastlick, University of Missouri.

*Embryology*

128. Some effects of mammalian hormonal substances on the differentiation of the gonads of an undifferentiated race of Rana catesbiana tadpoles. William O. Puckett, Princeton University.
129. Delayed fertilization and development of the guinea pig ovum through the sixteenth day of pregnancy. Richard J. Blandau and William C. Young, Brown University.
130. Comparative vertebrate hematology, H. E. Jordan, University of Virginia.
131. Model of a 2-mm. human embryo. J. L. Platt, University of Virginia. (Introduced by J. E. Kindred.)

*Physiology and Endocrinology*

132. Analysis of pituitary support of growth of body and viscera in pigeons. J. P. Schooley, O. Riddle and R. W. Bates, Carnegie Institution.
133. The interrelationship between retarded growth, aging and ultimate size of the body. C. M. McCay, L. A. Maynard, Gladys Sperling and Leroy Barnes, Cornell University.
134. Revival of organisms vitrified at 190°C. below zero. Basile Luyet, Eugene Hodapp and Gregory Thoennes, Saint Louis University.

### *General Morphology and Miscellaneous*

135. Tracing the path of spermatozoa through the substance of the frog's kidney by micro-injection of vasa efferentia. Roberts Rugh, Hunter College.
136. Microstomum feeding upon Chlorohydra. Wm. A. Kepner, M. A. Stirewalt and F. F. Ferguson, University of Virginia.
137. A monograph of the genus Macrostomum O. Schmidt 1848. Frederick Ferdinand Ferguson, University of Virginia. (Introduced by W. A. Kepner.)
138. Racial differences within the species Choas diffluens Müller, the common laboratory ameba. A. A. Schaeffer, Temple University.
139. A mosaic color pattern in the Mexican bean beetle. Nellie M. Payne, American Cyanamide Company.
140. The sealing of demonstration specimens in plastic containers. Elbert C. Cole, Williams College.
141. A low voltage lamp for general microscopic use. Elbert C. Cole, Williams College.
142. The plankton samples: a compact device for investigating quantitatively the distribution of zooplankton. George L. Clarke, Harvard University.

142 A. An experimental study of insect populations. Royal N. Chapman, University of Hawaii.

## THURSDAY EVENING, DECEMBER 29TH, 7.00 P.M.

*Zoologists' Dinner,* Winter Garden, Hotel Richmond

Address by Dr. F. B. Sumner, vice-president of Section F of the A. A. A. S. on *Human Psychology and Some Things that Fishes Do.*

(Open to all zoologists and friends.)

## FRIDAY MORNING SESSION, DECEMBER 30TH

*Academy of Medicine, 12th* and *Clay Streets*

This session will be held in four sections, as shown below.

Section A. *Symposium,* 9.30 A.M.; Auditorium, Academy of Medicine

### MATING TYPES AND THEIR INTERACTIONS IN CILIATE INFUSORIA; DETERMINATION, INHERITANCE AND RELATION TO SEXUALITY; WITH DEMONSTRATIONS

*H. S. Jennings, presiding*

(1) Introduction; with Demonstration. H. S. Jennings, The Johns Hopkins University.

(2) Paramecium aurelia; mating types and groups; lethal interactions, determination, inheritance, relation to natural selection. T. M. Sonneborn, The Johns Hopkins University.

(3) Paramecium bursaria: mating types and groups, mating behavior, self-sterility; their development and inheritance. H. S. Jennings, The Johns Hopkins University.

(4) Studies in conjugation in Paramecium caudatum and P. multimicronucleatum. A. C. Giese, Stanford University.
(5) Mating types in Paramecium caudatum. Lauren Gilman, The Johns Hopkins University.
(6) Mating types in Euplotes. Richard Kimball, Yale University.

Section B. *Cytology* and *Histology,* 9.30 A.M.; Room 10, Academy of Medicine

86. Recent studies on avian chromosomes. (5 min.) (Lantern.) Robert T. Hance, University of Pittsburgh.
87. New histological methods. (10 min.) (Lantern.) Robert T. Hance, University of Pittsburgh.
88. Some properties of living chromosomes. (15 min.) (Lantern.) John B. Buck and Robert D. Boche, Carnegie Institution of Washington, Baltimore.
89. Observations on abortive oocytes in the camel cricket. (10 min.) (Lantern.) M. W. Johnson, Duke University.
90. The structure of the Golgi apparatus in the tissues of amphibia. (15 min.) (Lantern.) Arthur W. Pollister, Columbia University.
91. A cytological study of transplanted hind limbs of chick embryos. (15 min.) (Lantern.) Herbert L. Eastlick, University of Missouri.
92. The effect of inhibitors of carbohydrate metabolism on the growth of chick fibroblasts. (15 min.) (Lantern.) Charles M. Pomerat, Clark University.
93. A new type of protoplasmic movement. (12 min.) (Motion picture.) W. J. Baumgartner, University of Kansas.
94. A quantitative study of the histology of the lymph nodes of the submaxillary region of 20-day-old albino rats. (15 min.) (Lantern.) J. E. Kindred, University of Virginia.
95. The duodenum of the turkey: histology and autodigestion. (15 min.) (Lantern.) Lauren E. Rosenberg, University of California. (Introduced by Charles A. Kofoid.)
96. Histology of the parotid gland of Bufo a. americanus Holbrook. (7 min.) (Lantern.) Paul L. Carroll and Charles G. Wilber, Marquette University.
97. Cross section anatomy of Anodonta grandis Say and Ostrea virginica Gmelin. (7 min.) (Lantern.) Paul L. Carroll, F. L. Mierzwa and H. J. Barnett, Marquette University.

Section C. *General Physiology,* 9.30 A.M.; Room 20, Academy of Medicine

98. Moulting in the crustacean, Crangon armillatus. (15 min.) (Lantern.) Hugh H. Darby, College of Physicians and Surgeons, Columbia University.
99. Chemical control of autotomy in Petrolisthes armatus. (10 min.) (Lantern.) H. H. Haskin and J. H. Welsh, Harvard University. (Introduced by A. C. Redfield.)
100. The birefringence of the sheath of the earthwworm giant nerve fibers. (15 min.) (Lantern.) G. Wellford Taylor, Washington University, St. Louis. (Introduced by Caswell Grave.)
101. Preliminary experiments on the color changes of the American chameleon, Anolis carolinensis (Cuvier). (10 min.) (Lantern.) F. H. Wilson, Tulane University.

102. The physiology of color change in the winter flounder, Pseudopleuronectes americanus. (12 min.) (Lantern.) Clinton M. Osborn, Harvard University. (Introduced by G. H. Parker.)
103. The illumination of the eye necessary for different melanophoric responses of Fundulus heteroclitus. (10 min.) (Lantern.) Earl O. Butcher, Hamilton College.
104. Reactions to light and the photoreceptors in certain decapod crustaceans. (10 min.) (Lantern.) Walter N. Hess, Hamilton College.
105. Some aspects of the relative roles of increased periods and constant periods of illumination in the sexual photoperiodic activation of the male starling. (15 min.) (Lantern.) J. Wendell Burger, Trinity College.
106. Effect of light and darkness on recovery in Peranema trichophorum after response to light. (15 min.) (Lantern.) Landrum B. Shettles, Johns Hopkins University.
107. Mechanisms involved in group protection of Euplanaria dorotocephala from ultraviolet radiation. (15 min.) (Lantern.) Janet Wilder, The University of Chicago. (Introduced by Frank R. Lillie.)
108. The influence of the pupillary light reflex upon dark adaptation measurements. (15 min.) (Lantern.) Charles Haig, Research Division for Chronic Disease, Welfare Island, and Department of Medicine, College of Physicians and Surgeons, Columbia University.
109. The yellow pigment of the Mexican bean beetle, Epilachna varivestris. Muls. (15 min.) (Lantern.) Nellie M. Payne, American Cyanamide Company.
110. The toxicity to honeybees of acid lead arsenate, calcium arsenate, and phenothiazine in relation to particle size. (10 min.) (Lantern.) Lloyd M. Bertholf, Western Maryland College and Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.

### Section D. *General Morphology and Miscellaneous,* 9.30 A.M.; Room 30, Academy of Medicine

111. Homology and anology. (12 min.) (Lantern.) Alan Boyden, Rutgers University.
112. Evolutionary allometry in the skeleton of the domesticated dog. (15 min.) (Lantern.) Hyman Lumer, Western Reserve University and Fenn College.
113. The ductus genito-intestinalis in the genus Castrada based upon Castrada lutheri n. sp. (5 min.) (Lantern.) William A. Kepner, M. A. Stirewalt and F. F. Ferguson, University of Virginia.
114. A method of excretion in Microstomum, O. Schmidt 1848. (5 min.) (Lantern.) M. A. Stirewalt, University of Virginia. (Introduced by Wm. A. Kepner.)
115. Grafting experiments directed toward ascertaining the location of factors of head regeneration in the earthworm. (15 min.) (Lantern.) Ben T. Painter, Harvard University. (Introduced by Herbert W. Rand.)
116. Regeneration and regulation of multiple heads in Planaria maculata and Planaria agilis. (10 min.) (Lantern.) Frank M. Hull, University of Mississippi.
117. Cannibalism in Planaria. (5 min.) (Lantern.) Frank M. Hull, University of Mississippi.

118. The food chain in a small Texan cave. (5 min.) (Lantern.) Lorus J. Milne, Randolph-Macon Woman's College.
119. Observation on some lower Turbellaria of the eastern United States. (10 min.) (Lantern.) E. Ruffin Jones, Jr., College of William and Mary.

## PAPERS PRESENTED BY TITLE

### *Embryology*

143. The development of Habrobracon eggs in different aqueous media. Elias M. Spotkov, Washington Square College, New York University. (Introduced by Ruth B. Howland.)
144. Observations on the karyokinesis during cleavage of the egg of the zebra fish (Brachydanio rerio). Edward C. Roosen-Runge, Brown University. (Introduced by J. W. Wilson.)
145. The development of the hypophysis cerebri in Cyprinus carpio. John Maxwell Robeson, Syracuse University. (Introduced by V. F. Lindeman.)
146. Production of various abnormalities in amphibian embryos by centrifugal force. C. S. Thornton, Kenyon College. (Introduced by E. G. Butler.)
147. Temperature tolerance and rates of development of the eggs of amphibians. John A. Moore, Columbia University. (Introduced by L. G. Barth.)
148. Reversal of polarization in the ear primordium. Edmund K. Hall, Department of Anatomy, University of Louisville.
149. Some effects of colchicine on the early development of the frog, Rana pipiens. Ralph Judson Bushnell, University of Georgia.
150. Tympanic membrane induction in anuran integument. O. M. Helff, New York University.
151. Development of tongue papillae in R. sylvatica. O. M. Helff and M. C. Mellicker, New York University.
152. Development of dermal plicae components of R. sylvatica. O. M. Helff and William Stark, New York University.
153. Growth rates of the hind limbs of Rana sylvatica during normal and induced metamorphosis. Campbell M. Bower, McMaster University. (Introduced by A. Emerson Warren.)
154. Effect of a respiratory stimulant on the development of the teleost, Oryzias latipes. A. J. Waterman, Williams College.
155. Form regulation in transplanted ear rudiments of the young chick embryo. A. J. Waterman and H. J. Evans, Williams College.
156. On the production of feather color pattern by mesodermal grafts between Barred Plymouth Rock and White Leghorn chick embryos. R. L. Watterson, University of Rochester. (Introduced by B. H. Willier.)
157. Development of embryonic gonad grafts in juvenile diamond-back terrapins. Paul L. Risley, State University of Iowa.
158. The embryogenesis of Drosophila following centrifuging at high forces. Herbert Shapiro, Princeton University and Clark University.
159. The temperature effective period for wing length of the pennant wing of Drosophila melanogaster. Fred Weinstein, Washington Square College, New York University. (Introduced by Morris H. Harnly.)
160. Parthenogenesis in termites. S. F. Light, University of California.

### *General Physiology*

161. The effects of methenamine, ethyl carbamate, and potassium cyanide on 'vital staining' of the chromatophores of the squid. Elbert C. Cole, Williams College.
162. A physiological solution for the lobster heart compared to isotonic glucose, urea and single salt solutions. William H. Cole and Louis A. Kazalski, Rutgers University.
163. Relationship between cyanide sensitivity and rate of respiration. Wilbur A. Robbie, State University of Iowa. (Introduced by J. H. Bodine.)
164. Respiration of Astasia sp. and Khawkinea halli. George E. Jay, Jr., State University of Iowa. (Introduced by T. L. Jahn.)
165. The effects of homologous carbamates on the respiration of blocked and active embryos. Guy M. Everett, State University of Iowa. (Introduced by J. H Bodine.)
166. The electrical response of the compound eye of the cecropia moth. Theodore Louis Jahn and Frederick Crescitelli, State University of Iowa.
167. Absorption of light in the Okoboji Lakes. A. B. Taylor and Theodore Louis Jahn, University of Illinois and State University of Iowa.
168. The electrical response of the grasshopper eye to colored light. Frederick Crescitelli and Theodore Louis Jahn, State University of Iowa.
169. The relation of the nervous system to skin potential in the intact frog. F. R. Steggerda and E. Ponder, University of Illinois and Cold Spring Harbor.
170. A study of the vitamin E deficiency effects in male and female rats reared on an iron treated ration. F. B. Adamstone and Edna M. Pratt, University of Illinois.
171. The hydrogen-ion concentration in the digestive tract of perch. Bertil G. Anderson, Western Reserve University.
172. The responses of the swimbladder of the guppy, Lebistes reticulatus, to sudden decreases in pressure. Frank A. Brown, Jr., Northwestern University.
173. Change in the coagulation time of blood of Galleria mellonella during metamorphosis. Ivon R. Taylor and Nathan Millman, Brown University.
174. The thickness of the limiting envelope of the rabbit erythrocyte. Francis O. Schmitt and David F. Waugh, Washington University.
175. Hydrogen-ion concentration of insect blood. Robert D. Boche and John B. Buck, Carnegie Institution of Washington, Baltimore.
176. The nature of the relation between the amount of closure and the size of the wound in the carapace of Daphnia. Bertil G. Anderson, Western Reserve University.
177. Occurrence of mitosis in diapause and postdiapause Melanoplus differentialis embryos. Theodore N. Tahmisian, State University of Iowa. (Introduced by J. H. Bodine.)
178. Bactericidal activity of 'royal jelly' of the honeybee. C. S. McCleskey and R. M. Melampy, Louisiana State University and Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.
179. The relation of imaginal population density to the postembryonic development of Tribolium confusum larvae. Thomas Park, Ella Virginia Miller and Catherine Z. Lutherman, The University of Chicago.

### *Cellular Physiology*

180. Pimelic acid as a growth factor for the ciliate, Colpidium campylum. R. P. Hall, New York University.
181. The origin and the function of the acid and the alkali in the food vacuoles in Paramecium and Amoeba. S. O. Mast, Johns Hopkins University.
182. Experimental studies on the longevity of unfertilized egg cells of Mactra solidissima. Victor Schechter, College of the City of New York.
183. Differences in the physical nature of the protoplasm of the Nereis egg following activation. Donald P. Costello, University of North Carolina.
184. The behavior of the oil drops in the centrifuged egg of Nereis limbata. Donald P. Costello, University of North Carolina.
185. Surface changes in the Arbacia egg upon fertilization. Robert Chambers, Washington Square College, New York University.
186. Effect of the hyaline plasma layer on coalescence of Arbacia eggs with oil-drops. M. J. Kopac, Washington Square College, New York University.
187. Further studies on the Devaux effect at oil-protoplasm interfaces. M. J. Kopac, Washington Square College, New York University.
188. The chemotactic response of the polymorphonuclear granulocytes to bacterial colonies. C. G. Grand, Washington Square College, New York University. (Introduced by M. J. Kopac.)
189. A cinematographic method of studying dispersion and concentration of chromatophoric pigment. Earle B. Perkins and William H. Cole, Rutgers University.
190. The effect of temperature on the size of the columnar cells of the mid-intestine of Japanese beetle larvae. Daniel Ludwig, University College, New York University.

### *Endocrinology*

191. A hormone affecting viability in crustaceans. Frank A. Brown, Jr., Northwestern University.
192. After-effects of pituitary injections upon the male genital system of Anolis carolinensis. Llewellyn Thomas Evans, Montana State University.
193. The effect of hypophysectomy on the testis of Fundulus. Samuel A. Matthews, Williams College.
194. Some effects of hypophysectomy in chick embryogenesis. Nicholas W. Fugo. State University of Iowa. (Introduced by Emil Witschi.)
195. Effect of pituitary implants on the mammary glands of hypophysectomized rats. Warren O. Nelson, Department of Anatomy, Wayne University, College of Medicine.
196. Effect of progesterone on the testes of hypophysectomized rats. Warren O. Nelson, Department of Anatomy, Wayne University, College of Medicine.
197. The response of the gonads of nestling passerines (Passer domesticus and Agelaius phoeniceus) to gonadotropic substances. Gardner M. Riley, State University of Iowa. (Introduced by Emil Witschi.)

198. Effect of theelin administration on female sparrows. A. R. Ringeon, University of Minnesota.
199. Effects of injections of epinephrine on the social order in small flocks of hens. W. C. Allee and Nicholas Collias, The University of Chicago.
200. Thyroidectomy and gonad response to gonadotropic hormones. Samuel L. Leonard, Rutgers University.
201. Effects of androgens on fish (Lebistes reticulatus). Wilburn J. Eversole and Robert Gaunt, Washington Square College, New York University.
202. Influence of sex hormones on sex differentiation in amphibia (Rana pipiens). Charles L. Foote, State University of Iowa. (Introduced by Emil Witschi.)
203. Hypertrophy of turtle thyroids induced by mammalian whole pituitary extract. Llewellyn Thomas Evans, Montana State University.
204. Studies on the reproductive tract of the male box turtle. Ira B. Hansen, The George Washington University.
205. Adrenal cortex and diabetes insipidus. Malvina Schweizer, Robert Gaunt, Warren O. Nelson and Eleanor Loomis, Department of Biology, Washington Square College, N. Y. University; Department of Anatomy, Wayne University; and Biological Laboratory, Cold Spring Harbor.
206. Effect of bilateral optic enucleation on certain reproductive phenomena in rats under various light conditions. Ludvig G. Browman, Montana State University.

### *Cytology and Histology*

207. A simple method for the sharp differentiation of the basophilic granules in the anterior pituitary. Sam R. Hall and John D. Hunt, Bureau of Dairy Industry, U. S. Department of Agriculture.
208. Possible evidence for a second type of basophile in the anterior pituitary of cattle. Sam R. Hall, Bureau of Dairy Industry, U. S. Department of Agriculture.
209. Effect of castration on the basophiles of the bovine anterior pituitary, with a consideration of the free-martin. Sam R. Hall, Bureau of Dairy Industry, U. S. Department of Agriculture.
210. The morphogenesis and cytogenesis of the pars buccalis in the duck pituitary. Ben T. Painter, Harvard University. (Introduced by A. B. Dawson.)
211. Cytological abnormalities in the testes of pituitary-colchicine injected rats. Opal Wolf, Goucher College.
212. Effects of low temperatures on salivary gland chromosomes. John B. Buck and A. M. Melland, Carnegie Institution of Washington, Baltimore.
213. Studies of imprint preparations of pigeon bone marrow. A. R. Ringoen, University of Minnesota.
214. A study of fibril formation in connective tissue, by the method of dissociation with potassium hydroxide. H. E. Jordan, University of Virginia.
215. Angle of capillary branching and endothelial valves. Benjamin W. Zweifach, New York University. (Introduced by Robert Chambers.)

216. Regeneration in the central nervous system of Fundulus heteroclitus embryos after cautery. S. M. Nabrit, Morehouse College. (Introduced by J. W. Wilson.)
217. Some observations on the histology of the digestive tract of the crawfish, Cambarus virilis Hagen. Arno F. Michaelis, Marquette University. (Introduced by Paul L. Carroll.)

### *Protozoology*

218. The nitrogen requirements of Lobomonas piriformis. Kenneth L. Osterud, New York University. (Introduced by R. P. Hall.)
219. Growth of Chlorogonium tetragonium at different hydrogen-ion concentrations. John B. Loefer, Berea College.
220. Effect of some 'excretory' compounds on growth of Chilomonas, Chlorogonium and Glaucoma. John B. Loefer, Berea College.
221. The selective action of inorganic media in bacteria-free cultures of Euglena. R. P. Hall and H. W. Schoenborn, New York University.
222. Coccidian infections of western painted turtles of the Okoboji region. Orville J. Deeds and Theodore Louis Jahn, State University of Iowa.

### *Ecology and Parasitology*

223. The role of temperature, drying and lethal factors in the hatchability of sexual eggs of Daphnia longispina and of Moina macrocopa. Thelma R. Wood, Brown University.
224. Development of eggs of the Japanese beetle (Popillia japonica Newman) near the threshold temperature. Henry Fox, University College, New York University. (Introduced by D. Ludwig.)
225. Number of eggs of oothecae of two introduced species of Asiatic mantids and the proportion hatching. Henry Fox, University College, New York University. (Introduced by D. Ludwig.)
226. Podagrion mantis Ashmead, an egg parasite of introduced Asiatic mantids. Henry Fox, University College, New York University. (Introduced by D. Ludwig.)
227. Food specificity of grasshoppers. F. B. Isely, Trinity University.
228. Observations upon concealing and warning coloration in the tropical rain forest. Frank M. Hull, University of Mississippi.
229. On the life cycle of a tapeworm, Diphyllobothrium sp., from the herring gull, Larus argentatus Pont. Lyell J. Thomas, University of Illinois.
230. The life cycle of the tyroglyphid mites infesting cultures of Drosophila melanogaster. Stanley G. Stolpe, State University of Iowa. (Introduced by Paul L. Risley.)

### *General Morphology*

231. Relative growth of the limb bones in the anthropoid apes. Hyman Lumer, Western Reserve University and Fenn College.
232. Arteriae coronariae (Cordis) in the higher primates. Charles F. De Garis and Ralph E. Chase, Department of Anatomy, University of Oklahoma, School of Medicine.
233. The growth of the parts of the urogenital system of the cat. Homer B. Latimer, University of Kansas.
234. Cross section anatomy of the hind limb of the frog. Wilfred F. Horner, Marquette University. (Introduced by Paul L. Carroll.)
235. The frontal clasper in the chimaeroid Callorhynchus. L. Hussakof, American Museum of Natural History.
236. The problem of cyclomorphosis in Daphnia. R. E. Coker, University of North Carolina.
237. Variations in color pattern in the black widow spider, Latrodectus mactans (Fabr.). Albert M. Reese, West Virginia University.
238. Carterius tenosperma (Potts), a species of fresh water sponge recently described as new to Wisconsin. James R. Neidhoefer, Marquette University. (Introduced by Paul L. Carroll.)
239. Additional North American cave planarians. Libbie H. Hyman, American Museum of Natural History.
240. New Anyphaenidae from Panama, with notes on known species. A. M. Chickering, Albion College.

# ABSTRACTS

An author index of abstracts begins on page 139.

## PAPERS READ

### 1. AN ANALYSIS OF LITHIUM MICROCEPHALY IN RANA PIPIENS. T. S. Hall, Yale University. (Introduced by J. S. Nicholas.) (Lantern; 10 min.)

Developmental abnormalities of the head, induced by lithiumizing the eggs of Rana pipiens and other amphibia, have been subjected by the author to three analytical procedures: 1) treating eggs with lithium salt solutions; 2) local application of such solutions to delimited regions on the surface of the early gastrula; 3) special microsurgical techniques. This report deals with results obtained by the first of these three methods.

By introducing the necessary variables and maintenance of appropriate controls, the work of earlier investigators has been checked, extended, and re-analyzed. It has likewise proved possible to modify the nature of the lithium effect by the addition of antagonistic bivalent cations to the culture media.

The most important observations, drawn from forty-two series of experiments, are: 1) lithium is effective in the production of microcephaly in developing embryos of Rana pipiens from shortly after the beginning of gastrulation until the time of closure of the neural folds; 2) the extent to which cephalic development is suppressed varies in a regular way with a) the concentration of lithium salt in the culture medium and b) the duration of treatment; 3) lithium chloride solution is more drastic in its effect than corresponding isotonic lithium sulfate solution; 4) if calcium is added, it is necessary to prolong the treatment required to produce a desired degree of abnormality to from two to five times the period required in ordinary calcium-free medium.

### 2. EXPERIMENTS ON THE TIME OF LOCALIZATION OF THE ORGANIZING CENTER IN THE EGG OF THE JAPANESE NEWT. J. Clark Streett, Jr., Princeton University. (Introduced by Ulric Dahlgren.) (Lantern; 15 min.)

The time of localization of the organizing center in the egg of Triturus pyrrhogaster was investigated by following the development of diploid egg fragments. These were produced by constricting the fertilized, unsegmented egg into two approximately equal parts by means of a hair loop, so that the female pronucleus and at least one sperm were isolated in one fragment. The plane of constriction was either axial, coinciding with the animal-vegetal axis, or equatorial, coinciding with the equatorial plane.

Of forty-three diploid egg fragments obtained by axial constrictions, thirty-six gastrulated, giving rise to normal or to slightly defective embryos. The other seven failed to gastrulate and apparently represent 'ventral embryos' (Spemann).

This result agrees with those obtained on the eggs of Triton and Triturus viridescens. Of seven fragments obtained by equatorial constrictions, all of the animal halves, which contained the diploid nucleus, failed to gastrulate. These nongastrulating fragments lived until the controls had completed gastrulation or reached the early neural plate stage.

The time of operation apparently had no influence on the results. In the axial constrictions it varied from 8 to 49 minutes after insemination, and in the equatorial constrictions, from 5 to 52 minutes after insemination. These experiments, though still incomplete, suggest that the organizing center in the egg of Triturus pyrrhogaster is early localized to one side of an axial plane, and probably below the equatorial plane.

### 3. The effects of extirpation of the notochord, undertaken at the medullary plate stage, in Amblystoma mexicanum. Irwin C. Kitchin, The Rice Institute. (Introduced by Edgar Altenburg.) (Lantern; 12 min.)

By extraction through an incision made into the archenteron (from below) the entire notochord may be removed in the neural plate stages without injury to the plate or other neighboring axial structures. The wound usually heals without any consequent malformations in the abdominal regions. The notochordectomized embryos develop heads which are completely normal. The trunk, however, fails to undergo normal stretching. This failure becomes increasingly obvious as the animals develop and is not compensated for by a thickening in the same region. The trunk and tail show no coordinated movement. The somites of the two sides may or may not fuse beneath the nerve cord. In cases where such a fusion takes place the metameric spinal ganglia show a tendency to follow the migrating muscle and may fuse in pairs along the midline. The axial cartilages do not show metameric development but form as a solid plate or rod beneath the nerve cord in that space normally occupied by the notochord. The nerve cord in cross section presents a normal picture indicating that those disturbances traced to defective substrate in the pre-medullary plate stages no longer take place if similar defects become present after the plate has been determined.

### 4. Metabolic properties of the regions of the amphibian gastrula. E. J. Boell, Yale University. (Introduced by G. A. Baitsell.) (Lantern; 15 min.)

The Cartesian diver ultramicromanometer method, devised by Linderstrøm-Lang, has been developed for the measurement of the anaerobic glycolysis, oxygen consumption, and respiratory quotient of the various parts of the amphibian gastrula.

With respect to the rate of anaerobic glycolysis, a substantial difference exists between the organizer region and the ventral ectoderm. But no significant difference exists between the magnitude of oxygen consumption in the two parts of the gastrula. However, there is a marked qualitative difference between the oxidative metabolism in the two regions for as soon as gastrulation begins the respiratory quotient of the dorsal lip region rises abruptly from a pre-gastrulation level of approximately 0.75 to unity. This high quotient is maintained throughout gastrulation and the formation of the neural folds. The respiratory quotient of ventral ectoderm, on the other hand, is much lower (0.75 to 0.85) and it is doubtful whether it ever reaches a value of unity before it is completely underlaid by ventral mesoderm.

## 5. The nature and mode of action of chemical evocators in embryonic induction in amphibia. C. H. Waddington, University of Cambridge, England. (Introduced by L. C. Dunn.) (Lantern; 15 min.)

One element in the complex activity of the primary organization center is the evocation of neural tissue. This reaction is produced by the chemical stimulation of gastrula ectoderm by the underlying mesoderm. Active substances are found in several types of crude extracts from the organization center and in fairly pure extracts consisting of non-saponifiable lipoids. Evocatory activity is shown by minute doses of ether-soluble hydrocarbons, etc., and by massive doses of some acids. The development of neural tissue can, however, also be initiated by other substances (e.g., methylene blue) which cannot be supposed to be related to the naturally occurring evocator.

It is suggested that the reason why the organization center is the only region of the living gastrula which is capable of evocation is because it has a particular metabolism which 'liberates' the evocator from some combined or masked form. Measurements have already revealed that several peculiarities of metabolism are in fact found in this region. A tentative working hypothesis ascribes the action of non-natural evocators to their ability to cause normal ectoderm to adopt the type of metabolism crucially characteristic of the organizer. It is not yet clear which of the organizer's metabolic peculiarities is responsible for the liberation of the evocator.

## 6. Modification of development in the sand dollar with NaCNS and Ca-free sea water. Olin Rulon, Wayne University. (Lantern; 15 min.)

Unfertilized and fertilized eggs of Dendraster excentricus were submitted to a range of concentrations of NaCNS in normal and Ca-free sea water for varying intervals of time with consistent results.

Unfertilized eggs in NaCNS (solution in sea water) show slight inhibition when fertilized and allowed to develop in sea water while similar eggs exposed to NaCNS plus Ca-free sea water (100 cc. Ca-free sea water plus 10 to 30 cc. 0.54 M NaCNS) for 12 to 24 hours give 'ectodermized' forms with reduced or no mesenchyme, gut or pigment when fertilized and allowed to develop in sea water. Fertilized eggs (1 to 2 cells) in NaCNS (100 cc. Ca-free or normal sea water plus 10 to 40 cc. 0.54 M NaCNS), with return to sea water after 12 to 24 hours, give a high percentage of mesenchyme filled exogastrulae ('endodermized' forms). Fertilized eggs (1 to 2 cells) in Ca-free sea water alone will develop approximately as far as the middle blastula stage before the cells begin to separate. Return to sea water at this time results in larvae with excess skeleton and arms.

The foregoing results are interpreted on the basis of a dynamic differential or gradient along the polar axis of the egg and early embryo. The 'ectodermized' forms appear to result from differential recovery while the 'endodermized' forms appear to be the consequence of differential inhibition of the more active or dominant region of the polar gradient.

### 7. DEVELOPMENT OF PARTS OF CHAETOPTERUS EGGS, WITH SPECIAL REFERENCE TO PARTHENOGENETIC MEROGONY. Ethel Browne Harvey, Princeton University. (Lantern; 15 min.)

The Chaetopterus egg can be broken by centrifugal force into two unequal halves of constant size, similar to the Arbacia egg. The non-nucleate half can be activated artificially (parthenogenetic merogone), but development proceeds only to the 2-cell stage. A similar slight development takes place also in the non-nucleate half when fertilized (fertilized merogone); the presence of a nucleus does not cause a much further development.

The nucleate half, both when fertilized and when activated artificially, may pass through the early cleavages according to the peculiar and characteristic annelid pattern. However, the egg tends to become amoeboid with large nuclei and irregular cells, quite similar to the whole egg both centrifuged and uncentrifuged when caused to develop parthenogenetically. Swimming blastulae have been obtained from the white (nucleate) halves, both fertilized and parthenogenetic.

### 8. THE EFFECT OF SHAPE ON THE POLARITY OF THE FUCUS EGG. D. M. Whitaker, Stanford University. (Lantern; 15 min.)

The Fucus egg is spherical. Immediately after fertilization it secretes a film of gelatinous pecten or cellulose-like material which gradually 'sets' to become a rigid cell wall. When a recently fertilized egg is gently drawn into a glass pipette of less diameter than the egg, and is then removed from the pipette, the egg remains elongated.

Eggs with long diameters 1.1 to 1.8 times short diameters were reared, each egg in a separate dish, in the dark at 15°C. in sea water at pH 7.8 to 8.2. Ninety-six per cent of 114 eggs formed rhizoids at or close to one end of the long axis of the cell, the more so the greater the elongation. The developmental axis is thus determined by the shape imposed on the cell.

Elongated eggs reared in sea water acidified to pH 6.0, on the other hand, tended to form rhizoids near the end of the short axis at the bottom of the dish. The bottom of the dish blocks diffusion of substances leaving (and entering) the egg, which results in a gradient. It has previously been shown that the egg responds very strongly to such gradients at pH 6.0 (forming the rhizoid on the side of greater concentration of substances diffusing from the same or from another egg), but hardly at all at pH 7.8 to 8.2. It is therefore supposed that in the present instance the shape effect is largely superseded by the gradient effect at pH 6.0.

### 9. RETARDATION OF CELL DIVISION BY IRRADIATION WITH ULTRAVIOLET LIGHT. Arthur C. Giese, Stanford University. (Introduced by C. V. Taylor.) (Lantern; 15 min.)

Retardation of the division rate (during early cleavage) of eggs of the sea urchin Strongylocentrotus purpuratus was greatest following irradiation with known dosages of radiations of λ 2804A, the next most effective wave-length of those tried being 2654A. Wave-length 3660A was without apparent effect even after large dosages, but slight retardation followed irradiation with λ 3130A.

For the early cleavages of eggs of Urechis caupo the retardation was observed to be greater following irradiation with λ 2804A than following irradiation with λ 2654A. For Paramecium caudatum similar results were obtained. In the latter case recovery from injury by λ 2654A was much slower than the recovery from injury by λ 2804A. The results suggest a selective effect of λ 2804A on the cytoplasm and a selective effect of λ 2654A on the nucleus.

### 10. A POSSIBLE EXPLANATION OF HOW X-RAYS MAY PRODUCE BOTH DEATH AND MALIGNANT GROWTH IN CELLS BASED ON BOVERI'S THEORY OF CANCER AND CERTAIN EXPERIMENTAL OBSERVATIONS. Paul S. Henshaw, Memorial Hospital, New York City, and National Cancer Institute, Washington. (Lantern; 15 min.)

Boveri in 1914 postulated a theory of carcinogenesis based upon the idea of chromosome imbalance, the inspiration for which came from the observation of abnormal chromosome distribution in Arbacia eggs following the production of supernumerary attraction systems (or asters) by polyspermy. Accessory poles cause the chromosome complexes intended for two cells to be distributed among more than this number and accordingly produced cells deficient in chromosome contents. Boveri's idea was that the loss of certain restraining influences by loss of chromosomes allowed the cells in specific instances to manifest malignant behavior.

Whether the loss must be as great as whole chromosomes or whether a restraining influence is lost rather than a stimulating influence gained may be questioned in the light of more recent development, but the broad principle of chromosome (or genic) imbalance is one of the plausible theories of cancer at the present time.

Recently it has been our privilege to irradiate Arbacia punctulata gametes with x-rays and observe the effects on development following fertilization. Among other things noted was a high frequency of multipolar cleavage (nearly 100% when the treatment is adequate) resembling in close detail that produced by polyspermy. Investigating this it was found that the sea urchin blastomeres resulting from x-ray induced multipolar cleavage were no exception to the rather general finding that the cell progeny produced by multipolar cleavage is doomed to an early disintegration.

Thus, since multipolar cell division usually causes cells to die, the observation that this kind of division may be caused by x-rays has a very interesting relationship to the Boveri theory, for it offers a possible explanation of how x-rays by the same mechanism of action may produce both death and malignancy in cells —seemingly very opposite types of responses of cells to radiation but nevertheless well known to occur.

### 11. EXPERIMENTAL STUDIES ON THE REPRODUCTIVE SYSTEM OF THE MALE ANURAN. Roberts Rugh, Hunter College. (Lantern; 15 min.)

A morphological and physiological study of the reproductive tract of the male anuran reveals that (in Rana pipiens) about 2% of the malphigian corpuscles (total more than 2000) are involved in sperm transfer to the wolffian duct during sexual activity. Thus study was made by micro-injection of India ink into the vasa efferentia and tracing the ink as it passes into the malphigian corpuscles, through the related uriniferous tubules and into the ureter. This was confirmed by anterior pituitary hormone stimulation of hibernating males and histological tracing of spermatozoa from their Sertoli cells, within the seminiferous tubules, until they appear in the wolffian duct (ureter). There is no evidence, either histological or by micro-injection methods, of any trace of a Bidder's canal traditionally described as a sperm duct leading posteriorly along the mesial margin of the kidney to join the wolffian duct after it leaves the kidney. The spermatozoa enter the urinary tract at the point of the malpighian corpuscle and then are carried along by truly urino-genital ducts.

### 12. AN ANALYSIS OF GONADAL WEIGHTS IN ADULT TRITURUS VIRIDESCENS IN RELATION TO THE ANNUAL REPRODUCTIVE CYCLE. A. Elizabeth Adams, Mount Holyoke College. (Lantern; 15 min.)

In order to study the condition of the reproductive tract of the adult, aquatic form of Triturus viridescens of both sexes throughout the year, newts were collected from one locality at intervals of 2 to 4 weeks in parts of several years. Data on size (length and body weight) and on secondary sex characters and weight of gonads were secured and the gonads, as well as parts of the reproductive tract, were studied histologically. Graphs of absolute gonadal weights, of gonadal weights as percentages of body weights and, in males, of weights of immature and mature sections of the testis have been made. The greatest weight of the testis occurs in summer when active spermatogenesis is in progress. In early fall, during the fall mating season, the weight of the whole testis decreases as evacuation of the spermatozoa from the newly 'matured' portion occurs. The weight of the ovaries is least in the summer and not until early fall does their size suddenly rise to a maximum, as yolk is laid down in the oocytes. During the winter, gonadal size in both sexes varies relatively little from its condition in the fall. In the spring breeding season, the weights of gonads in both sexes decrease as eggs and spermatozoa are shed. The analysis of the gonadal weights as well as the histological study of the gonads indicates that there is one gametogenic cycle in each year.

### 13. THE PARS TUBERALIS OF THE HYPOPHYSIS IN TOADS. Wayne J. Atwell, University of Buffalo. (Lantern; 15 min.)

Five species of toads have been studied with special reference to the form and location of the pars tuberalis of the hypophysis cerebri. These include the North American toads Scaphiopus holbrookii (Harlan), Bufo fowlerii (Hinkley), and B. valliceps (Weigmann), the South American toad B. arenarum, and the South African toad, Xenopus laevis.

In each of the above species the pars tuberalis was found to consist of a pair of discrete epithelial plaques closely applied to the brain floor and located cephalad to the remainder of the gland. This is contrary to Rimer who did not find "the characteristic tuberalis elements of other Anura in the gland of Xenopus." Instead, a mass of basophilic cells which projects forward from the anterior lobe in the midline is identified as the pars tuberalis. Rimer concluded that "the pituitary gland of Xenopus . . . . essentially conforms to the salamandrine type in the incomplete separation of the pars tuberalis from the pars anterior."

The central mass of basophilic cells described by Rimer is readily found but the separate epithelial plaques are also present. These findings necessitate a different interpretation of the work of Hogben and Slome on color changes in toads versus frogs following removal of the anterior lobe.

Interesting species differences in the infundibulum and the tuber cinereum also have been observed.

### 14. AUGMENTATION OF PITUITARY EXTRACT BY CHLOROPHYLL PLANT GROWTH HORMONES AND HEMIN. W. R. Breneman, Waterman Institute and Department of Zoology, Indiana University. (Lantern; 10 min.)

White Leghorn cockerels and 23-day-old female rats were injected with pituitary gonadotropic hormone after admixture in vitro with alcohol or water-soluble chlorophyll, plant growth 'hormones' (indole-butyric acid or 1-napthalene acetic acid), or hemin. The action of the pituitary extract was augmented in both the cockerel and rat series. The maximum response was observed in those animals injected with the chlorophyll-pituitary mixtures. The plant growth hormones produced smaller but quite definite increases in ovarian weights of the rat. Hemin added to the pituitary extract had no augmenting effect, but after the addition of a small amount of pyridine to the mixture the action of the hormone was augmented.

Cockerels were given a single injection when only 1 day old and were without food or water for 72 hours at the end of which time they were killed. The response of the testes to the various admixtures of hormones and augmenting substances was similar to that observed for the female rat. Testes of chicks injected for 5 days, however, either from the fifth to ninth or from the tenth to fourteenth days, did not evidence the augmentation response. A total of 137 rats and 243 chicks were used in these experiments.

### 15. THE MORPHOLOGY OF THE THYROID GLAND IN THE METAMORPHOSING RANA PIPIENS. Savino A. D'Angelo and Harry A. Charipper, Washington Square College, New York University. (Introduced by Morris H. Harnly.) (10 min.)

Larvae of Rana pipiens, obtained by Rugh's method of ovulation, were reared through metamorphosis in order to make a detailed morphological study of the thyroid gland in selected stages of the metamorphic process. The glands were seen to migrate in a caudad and laterad direction along the ventral surface of the hyoid cartilage, the path traversed being that of an inverted 'Y.' A relatively close relationship of the gular muscles to the thyroid lobes was maintained throughout the early stages (25 mm. total length, hind limb bud, 3 mm. hind limbs, fully differentiated hind limbs), whereas, in the later stages (fore limb emergence and tail resorption) none of these structures was in the immediate vicinity of the glands.

Judged by the height of the epithelium, the collapsed follicles, the diminished and vacuolated colloid, and the vacuolated cytoplasm, the maximum activity of the thyroid gland occurred at the period of tail resorption. A progressive increase in folliculation and amount of colloid characterized the period between the early hind limb and fore limb emergence stages.

The Gogli apparatus was observed to undergo marked morphological change as metamorphosis proceeded. In the early stages, the Golgi substance appeared as a small compact configuration which was replaced in the later stages by a highly elaborated network which assumed either a filamentous or ring-like form.

### 16. The 'critical' period in the metamorphosis of Rana sylvatica. Savino A. D'Angelo, Albert S. Gordon and Harry A. Charipper, Washington Square College, New York University. (Lantern; 10 min.)

A period has been determined in the development of Rana sylvatica at which nutritional and hormonal factors are sufficiently established to permit larvae, unfed and reared in distilled water, to complete their metamorphosis. This 'critical' period occurs between the 3 mm. and 8 mm. hind limb length stages, for larvae fed normally to the former stage and then starved make little or no progress in metamorphosis, whereas tadpoles fed normally to the 8-mm. hind limb stage and then starved, become frogs. Histological examination of the thyroids in these non-metamorphosing animals reveal peculiar glands, which, despite possessing a vacuolated colloid, appear otherwise inactive. The pituitary glands of this group appear less differentiated than those of normal metamorphosing controls in which sudden pituitary differentiation and thyroid growth occur at the time of the 'critical' period.

Nutritional factors were investigated by administering various diets to 3 mm. hind limb larvae maintained in distilled water. A first group receiving solely a basic diet of rolled oats (low iodine content) was retarded in development. A second group given the rolled oats plus heads severed from 3 mm. hind limb animals metamorphosed precociously. Another group fed the decapitated larvae together with the rolled oats diet metamorphosed at the same rate as a control group receiving liver and spinach.

Such experiments indicate that the thyroids of 3 mm. hind limb larvae possess active principle. Consequently, the inability to metamorphose in inanition may be associated with failure of a mechanism, presumably the pituitary, governing the release of thyroid secretion.

### 17. The reticulo-endothelial system and hormone refractoriness. IV. The antithyrotropic complex. Albert S. Gordon, Savino A. D'Angelo and Harry A. Charipper. (Lantern; 15 min.)

Young normal and splenectomized male guinea pigs were given daily subcutaneous injections of 5 mg. thyrotropic hormone (Rowlands and Parkes extract) for 92 days. The splenectomized animals, in addition, received 1 cc. of 1% trypan blue intraperitoneally beginning with the fifteenth day after splenectomy and every second day thereafter, to prevent the remaining reticulo-endothelial elements from producing the antithyrotropic principle. The effects of powder preparations (Zondek and Sulman) of the plasmas obtained from these two groups of animals were tested in Rana pipiens tadpoles and young guinea pigs.

When injected alone into tadpoles, both types of plasma accelerated metamorphosis as judged by rate of hind limb growth and thyroid histology, the plasma from the thyrotropic injected 'dye-blocked' splenectomized guinea pigs accelerating metamorphosis more than that obtained from the normal injected guinea pigs.

The latter plasma, however, was found to inhibit simultaneously injected thyrotropic hormone in tadpoles, whereas that obtained from 'dye-blocked' splenectomized animals exerted no appreciable inhibition.

Using thyroid weight, histology and metabolism as criteria for thyrotropic effect, similar results were found using the young guinea pig as the test animal.

The acceleratory effects obtained when the plasmas are injected alone can be explained by assuming that the antithyrotropic complex dissociates. The results show also that production of inhibitory principle depends on reticulo-endothelial activity and that splenectomy, coupled with 'dye-blocking,' decreases the production of antithyrotropic principle presumably because of the partial removal of reticulo-endothelial cells and the interference with the activity of the remaining elements.

### 18. The production of experimental exophthalmos and the effect of thyroxin and other iodine compounds on its development.

George K. Smelser, Columbia University. (Lantern; 15 min.)

The exophthalmos produced by injection of anterior pituitary extracts into thyroidectomized guinea pigs closely resembles that found clinically in post-thyroidectomy cases. The orbital contents of exophthalmic animals are heavier, and the muscles and particularly the fat are edematous. Biopsies of orbital fat and muscles from clinical cases of marked exophthalmos reveal a comparable edematous infiltration of all tissues. Histologically the infiltration is identical in the human and experimental cases.

The effect of concurrent injections of sodium iodide, diiodotyrosine and thyroxin on the development and degree of exophthalmos produced by anterior pituitary administration was determined. Such exophthalmos is unaffected by coincident administration of either sodium iodide or diiodotyrosine. Thyroxin injections, however, reduce the degree of exophthalmos produced by anterior pituitary extracts and the incidence of its occurrence in hypothyroid animals from 86% to 52%. The weight of the orbital contents is increased by pituitary extract injections over thyroxin treated controls, but not above that of uninjected animals. Thyroxin injections alone produce a definite enophthalmos and ptosis which is accompanied by a marked reduction in the weight of the orbital contents. All orbital tissues are involved in this decrease.

These experiments offer an interesting correlation with clinical cases which are most severe in hypothyroid patients following thyroidectomy. Treatment of such cases with iodides has been ineffective whereas, in a few instances, thyroid administration relieved the condition.

### 19. Effect of bilateral optic enucleation on body weights of rats under various light conditions.

Ludvig G. Browman, Montana State University. (Lantern; 15 min.)

Male and female rats which have had both eyes removed on the day of birth and have been kept for the first 100 days of life 1) under normal colony conditions, 2) in continuous darkness, and 3) in continuous light, have growth curves that are consistently lower than those of their normal litter mates of the same sex. These sub-normal growth curves are first apparent when the animals are about 25 to 30 days of age, and occur in all enucleated animals irrespective of light conditions.

The retardation of growth occurred only when the rats were enucleated before the age of 20 days. When they were enucleated at 20 days of age they had a growth curve only slightly lower than non-enucleated litter mates, whereas animals enucleated at 30 days or more had essentially the same growth curves as normal litter mates of the same sex.

These experiments and other unreported ones suggest that the eye may elaborate a 'principle' which is essential for normal growth. It is possible that this 'principle' may act via the pituitary to produce its effect.

### 20. ON THE NATURE AND ACTION OF DIANTLIN, A NEW HORMONE CARRIED BY THE SPERMATOZOA OF THE OYSTER. T. C. Nelson and J. B. Allison, Bureau of Biological Research, Rutgers University. (Lantern; 15 min.)

A hormone causing increased water flow in the oyster, reported by Nelson, designated diantlin (Gr. diantlo, to pump through) is not secreted in stimulating concentrations into sea-water in true or colloidal solution. It cannot be separated from the spermatozoa of the oyster by dialysis through collodion membranes, by filtration, or by centrifugation. It is an integral part of their cellular structure and is associated with living or dead spermatozoa or with insoluble fragments thereof. If oyster spermatozoa are fragmented by freezing, hypertonic sodium chloride or ammonium sulfate solutions, or by distilled water, insoluble protoplasmic constituents containing protein and diantlin are liberated. On standing a few hours or after digestion by trypsin for a few minutes some of the insoluble protein liquefies and potency is lost, though ruptured membranes of spermatozoa remain. Trypsin, however, does not destroy the activity of diantlin on intact spermatozoa. If the soluble material is washed free from water soluble substances, liquefaction does not occur as rapidly on standing and the activity of the preparation is retained. Washing probably removed most of the water soluble intracellular proteolytic enzymes liberated from broken cells, particularly from the leucocytes which often contaminate suspensions of spermatozoa. Diantlin is inactivated by heat and other protein denaturents. These facts indicate that diantlin is a protein, carrying specific reactive groups, which is a part of living oyster spermatozoa and carried by them to the receptors present on the gills. Like an enzyme, denaturation or hydrolysis of the protein destroys the activity.

### 21. SEX CHANGE AND PHYSIOLOGICAL INTERSEXUALITY IN OSTREA VIRGINICA. Paul S. Galtsoff. U. S. Bureau of Fisheries. (Lantern; 15 min.)

Sex change in O. virginica was demonstrated on individually marked oysters whose sex was determined by inducing spawning, ascertaining the discharged products and obtaining kymograph records of spawning reaction of each. During the first year (1937) 8.0% of males changed to females and 13.1% of females changed to males, total change in either direction being 9.7%. In second year 11.2% of males changed to females and 12.1% of females became males, total percentage of changes in either direction being 11.5. Sex reversal among oysters already having changed their sex occurs much more often. Out of the total of thirty-two oysters which changed sex any time between 1936 and 1938, twenty-two (69%) reversed their sex. The results suggest presence in the population of a group of animals with unstable gonads.

Development of spawning reactions, different in the two sexes, and characterized in females by the rhythmicity of muscular contractions and discharge of eggs through the gills, fails sometimes to keep pace with gonadal changes and results in physiological intersexuality. Some of the sex reversed males, possessing ripe ovaries, reacted in typically male fashion discharging eggs through the cloaca. A complete female spawning reaction occurred, however, at the end of the reproductive season when the intersexuality disappeared.

### 22. On the role of certain hormones in organic synthesis. F. H. Pike and R. L. Zwemer, Columbia University. (Lantern; 10 min.)

In animal physiology much attention has been directed toward hormone activity in stimulating cells to mobilize stored materials, secrete, contract and otherwise use up reserves. The purpose of this communication is to draw attention to the equally, or even more, important function of hormones in stimulating the synthesis of biological compounds which would not form spontaneously, in the thermodynamic sense of the term. Insulin is associated with glycogen formation. The parathyroid hormone acts to get calcium recombined in skeletal muscle, and in the absence of the hormone, calcium liberated during muscular activity fails to recombine and is excreted. Similarly, potassium-lipid which exists in nervous tissue and potassium-phosphocreatine in muscle may fail to be recombined after nervous and muscular activity in the absence of adrenal cortex.

We would express the view that certain hormones are chemopoietic agents, in contrast to the chemoclastic agents which have been emphasized most strongly.

### 23. Sequence of appearance of x-radiation effects. Titus C. Evans, State University of Iowa. (Lantern; 8 min.)

Studies have been made of the effects of x-radiation on the eggs of Melanoplus differentialis at different stages in the development of the embryo. Effects have been noted on subsequent growth, cellular injury, respiration and weight.

It is apparent from the results obtained that the differentiation of cells into an organized embryo is necessary for the usual increase in oxygen consumption and water intake of the egg as indicated by increase in weight.

### 24. Effects of roentgen rays on the activation and production of the enzyme tyrosinase in the insect egg (Orthoptera). O. Malcolm Ray, State University of Iowa. (Introduced by Titus Carr Evans.) (Lantern; 7 min.)

The effects of roentgen rays on the activity and production of tyrosinase have been studied. The activity of the enzyme was determined manometrically by the standard Warburg apparatus at 25°C. Tyramine-HCl was used as a substrate and the enzyme was activated with sodium oleate to detect any destruction of activity by irradiation.

Tyrosinase extracts prepared from grasshopper eggs were unaffected by doses of from 500 to 5000 r. Neither activation nor inhibition of the enzyme was observed up to 10 days after irradiation.

Exposing diapause eggs to 10,000, 20,000 and 30,000 r did not alter the activity of their tyrosinase although the embryos were killed. Similar results were obtained with postdiapause eggs regardless of whether they were permitted to develop or were kept inactive by placing at 0°C.

The exposure of 1- to 5-day-old eggs to 1000 r showed that the susceptibility to roentgen irradiation decreases markedly in the first few days after laying. Whereas, 1000 r on the first day prevent tyrosinase formation, 30,000 r on the second day reduce it only to about 50% of the control concentration. One thousand r on 5- to 7-day-old eggs kill all of the embryos but do not affect the subsequent production of tyrosinase.

The tyrosinase-producing cells are unique in that doses of 60,000 to 180,000 r at the time of their greatest activity will not prevent them from completing their function.

### 25. ONTOGENY OF THE NATURAL ACTIVATOR OF THE ENZYME, TYROSINASE. J. H. Bodine, O. Malcolm Ray and Thomas H. Allen. State University of Iowa. (Lantern; 7 min.)

A quantitative study has been made of the relative activity and amount of the naturally occurring activator of the enzyme tyrosinase in the egg of the grasshopper, Melanoplus differentialis, during its entire embryonic development. By means of centrifugal force, it is possible to separate activator from eggs of all ages and to test its potency on inactive enzyme preparations from eggs of different ages. All possible combinations of activator and inactive enzyme can thus be made.

Marked changes in the potency of the activator occur. No correlations seem to exist between the presence of inactive enzyme and activator since in young eggs no enzyme is present while activator of highest potency is found. Also there seems to be no direct proportionality between potency of the activator and volume of the centripetal layer from which it is derived.

### 26. THE EFFECT OF PYOCYANIN ON THE RESPIRATORY MECHANISM OF THE GRASSHOPPER EMBRYO (MELANOPLUS DIFFERENTIALIS). Loren D. Carlson, State University of Iowa. (Introduced by J. H. Bodine.) (Lantern; 8 min.)

The stimulation of the oxygen consumption of diapause (inactive) and post diapause (active) embryos by pyocyanin was determined manometrically. The most effective concentration of pyocyanin was found to be 0.02% and consistently caused greater increase in the oxygen consumption of the diapause embryos.

Since the action of the pyocyanin is said to be involved in the dehydrogenase-cytochrome-oxidase system, the effect of three inhibitors; cyanide, 0.001 M; carbon monoxide, 95%; and urethane of narcotic concentration (0.025 M and 0.05 M) was tested on this pyocyanin stimulation. Only in the case of carbon monoxide and stronger concentrations of urethane was this stimulation at all reduced and then never more than 30%. Combinations of carbon monoxide and urethane showed that the effect of these substances was not additive.

The pyocyanin seems to be acting independently of the dehydrogenase, cytochrome or oxidase providing the specificity of the inhibitors used leads to differentiation of the Keilin system.

27. RESPIRATORY QUOTIENTS OF THE FEMALE HONEYBEE DURING LARVAL AND PUPAL DEVELOPMENT. R. M. Melampy and E. R. Willis, U. S. Department of Agriculture and Louisiana State University. (Lantern; 12 min.)

The results of a biochemical study of the growth of the reproductive and worker castes of the female honeybee (Apis mellifica L.) seemed to indicate that caste differentiation is associated with metabolic activity during larval and pupal development. The queen or reproductive caste is characterized in the larval stage by a more rapid growth rate as well as a higher nitrogen, lipid, carbohydrate and energy content when compared to the workers. The metabolic rate accompanying the differentiation of the female larvae into queens and workers was determined using the Warburg procedure. During the larval period the R.Q. was greater than unity for both castes, indicating the synthesis of lipid. The R.Q. was between 0.80 and 0.90 for the pupal stage of the queen whereas the worker values were between 0.90 and 1.00 during metamorphosis.

28. PIGMENT METABOLISM STUDIES. Frank H. J. Figge, Department of Anatomy, University of Maryland Medical School. (Introduced by Eduard Uhlenhuth.) (Lantern; 15 min.)

The indophenol dyes alter the pigment metabolism of larval Amphibia. The study of the action of these dyes was undertaken to discover some of the intracellular factors that regulate the known chemical reactions and enzymes involved in melanin formation. In this study, these facts have been established: There was a great variability with regard to the effectiveness of the dyes in producing pallor in different species. They were most effective in Necturus, a form with low metabolism and which resembles the relatively non-pigmented cave salamanders. The dyes eliminated melanin in hypophysectomized animals. The rate of dye decolorization bore no relation to the rate of pigment destruction. The action of the dyes was inhibited by cold and was not photodynamic. The dyes were not effective in dead animals, in large animals, nor when injected intraperitoneally. Pigment removed from cells was not decolorized by the dyes.

The dyes did not destroy, but accelerated the action of dopa oxidase, the enzyme theoretically responsible for production of melanin in vertebrates. The dyes produced the pallor by inhibiting the enzyme tyrosinase and thus prevented the formation of melanin. The evidence for the last statement will be given and its significance will be discussed.

29. THE AFFINITY OF HEMOGLOBIN FOR OXYGEN IN MARINE FISHES. F. G. Hall, Duke University. (Lantern; 8 min.)

Affinity of hemoglobin of marine fishes for oxygen varies greatly in different species. There appears to be a correlation of hemoglobin function with activity and habitat of fishes. Active fishes having bloods with high oxygen capacities seem to possess hemoglobins with low affinities for oxygen and less active fishes with low oxygen capacities have hemoglobins with relatively high affinities. Variations in hydrogen ion concentrations seem to have a greater effect upon the hemoglobins of some fishes than others. Hemoglobins of sharks and skates are little affected by changes in pH. While those of mackerel, butterfish, menhaden, etc., are markedly affected.

### 30. THE ROLE OF HEMOGLOBIN IN AMPHIBIAN DEVELOPMENT AND METAMORPHOSIS. F. H. McCutcheon, North Carolina State College. (Introduced by F. G. Hall.) (Lantern; 7 min.)

During the life history of the bullfrog the oxygen dissociation curve of its hemoglobin was found to undergo changes which followed a definite trend. The curves shifted from left to right during metamorphosis. Changes in the hemoglobin seem to be correlated with the physiological conditions of the animal and with the loci of erythrocytopoiesis.

### 31. THE EFFECT OF $CO_2$ ON THE $O_2$-COMBINING POWER OF WHOLE AND HEMOLYZED MARINE FISH BLOOD. R. W. Root, E. C. Black and Laurence Irving. College of the City of New York and Swarthmore College. (Lantern; 15 min.)

Oxygen dissociation curves for both whole and hemolyzed blood of three marine fishes, namely, the sea robin, tautog and toadfish, under comparable $CO_2$ tensions have been obtained, as well as curves showing the relation between loss in $O_2$ capacity and $CO_2$ tension. The $O_2$-combining power of whole blood is markedly reduced by only 25 mm. $CO_2$. The $O_2$-combining power of hemolyzed blood, on the contrary, particularly that of the tautog and toadfish, is practically unaffected by $CO_2$, being nearly the same at 25 mm. $CO_2$ as at 0 to 1 mm. $CO_2$ tension. The oxygen combining power of sea robin blood is less affected by hemolysis than the blood of the others, although the $O_2$ combining power, in the presence of $CO_2$ is definitely increased after hemolysis. In the light of these findings the conclusion is reached that the factors determining the equilibrium between fish hemoglobin and $O_2$ in whole blood, particularly in the presence of $CO_2$, depend upon the structural integrity of the corpuscles. The hemoglobin itself may or may not be sensitive to $CO_2$ as it is reported for mammalian blood, but the major effect of $CO_2$ on fish blood operates through some factor or condition, like permeability, which depends upon the organization of the red cell.

### 32. OXYGEN TRANSPORT BY THE BLOOD OF THE CARP AND THE COMMON SUCKER. E. C. Black, University of Pennsylvania and Swarthmore College. (Introduced by Laurence Irving.) (Lantern; 15 min.)

A study has been made of the ability of the blood of the carp (Cyprinus carpio L.) and the common sucker (Catastomus commersonii Lacépède) to transport oxygen. Measurement of the effect of carbon dioxide upon this transport capacity has shown marked differences between the blood of these fish. The carp can oxygenate its blood at pressures of carbon dioxide which would render the blood of the sucker useless for respiratory purposes. On the other hand, the blood of the sucker could unload oxygen at a given tissue pressure of carbon dioxide far lower than that of the carp. These facts are discussed in relation to their significance to the total respiratory picture.

The relation of the effect of carbon dioxide upon oxygen combining power of the blood indicates that respiratory transport in certain fish is extremely sensitive to carbon dioxide. Fish vary greatly in this respect.

33. THE INFLUENCE OF CARBON DIOXIDE ON THE UTILIZATION OF OXYGEN BY CERTAIN SPECIES OF FISH IN ALGONQUIN PARK, ONTARIO. F. E. J. Fry, Ontario Fisheries Research Laboratory and University of Toronto and E. C. Black, University of Pennsylvania and Swarthmore College. (Introduced by Frank R. Kille.) (Lantern; 15 min.)

The ability of fishes to utilize oxygen in the presence of carbon dioxide varies to a marked degree from species to species, and to a considerable extent from individual to individual within the range of a single species. This variation has been measured for a number of species in Algonquin Park, Ontario. It is closely correlated with the summer habitat of these fish. Fish living in shallow water among aquatic vegetation are least sensitive.

It is believed that this behavior of the intact fish with respect to concentrations of dissolved carbon dioxide is related to the ability of the blood to hold oxygen in the presence of carbon dioxide.

34. METABOLISM STUDIES OF THE DEVELOPMENT OF TEMPERATURE REGULATION IN BIRDS. S. Charles Kendeigh, Baldwin Bird Research Laboratory and University of Illinois. (Lantern; 10 min.)

The rate of metabolism of nestling house wrens of ages 0, 3, 6, 9, 12 and 15 days was measured at air temperatures of 105, 100, 95, 90, 80 and 70° F. and below by collecting the carbon dioxide output in a modified Haldane apparatus over a period of 3 hours in each experiment. Variations in rate of heart beat and intensity of muscle tremors were measured by piezo-electric crystals. Correlations are made between body temperature, carbon dioxide output, water loss, weight loss, activity, rate of heart beat, and muscle tremors. The development of temperature regulation is a gradual one in these altricial birds, beginning soon after hatching and not reaching full perfection until after the autumn molt. The respiratory quotient averages 0.73 for all ages except 3 and 6 days when it is 0.84 and 0.82 respectively. Rate of heart beat at different air temperatures varies in the same manner as carbon dioxide output. Water loss increases slowly with rise in temperature in ages up to 9 days, when temperature control becomes established, after which there is no change with temperature up to 100°. In all ages, water loss at 105° is approximately double what it is at 100°, due to increased breathing and body temperature. Variations in heat production, as measured by carbon dioxide output, agrees with variations in intensity of muscle tremors at all temperatures up to at least 100° and a causal relation may exist between the two.

35. CYCLIC VARIATION IN THE HEART RATE OF WILD BIRDS. Eugene P. Odum, Baldwin Bird Research Laboratory and University of Illinois. (Introduced by S. C. Kendeigh.) (Lantern; 5 min.)

By the use of a piezo-electric crystal attached to a perch as a pickup unit and a crystal-driven pen writing on moving paper as a recorder it has been possible to determine heart rate and to a lesser extent respiration rate and muscular activity under a wide variety of conditions with a minimum disturbance to the bird. Heart rate determinations of about twenty-five species (mostly passerines) and 200 individuals both adults and nestlings have been made under controlled laboratory conditions and in the field at the nest.

The heart rate of small birds may be anywhere from 170/minute to 1060/minute depending on the species and conditions. In general the smaller birds have a faster rate but the correlation is not absolute. Variations in rate are marked and rapid; the sensitivity of the heart and its adjustment to varying conditions are remarkable. In addition to variations resulting from specific stimuli a definite cyclic rhythm is apparent in most species and individuals, being especially marked under standard (basal) conditions. As many as fifteen cycles may occur in a minute and variation in a cycle may be 10 to 20% of the average for the minute period. Consequently, an isolated reading means very little; to get a true picture it is necessary to plot the rates at short intervals (10 seconds) over as long a period as possible.

### 36. CHEMICAL MEDIATORS AND THE HEART OF PANULIRUS ARGUS. John H. Welsh, Harvard University. (Lantern; 10 min.)

The isolated heart of the spiny lobster of the West Indies continues to beat for many hours when properly perfused with an artificial medium. The various effects of eserine, atropine and acetylcholine (ACh) all indicate that the latter substance plays an important role in normal maintenance of the beat of this heart. Eserine 1 in $10^5$, which protects ACh from destruction by choline esterase, may restore a normal beat after a period of irregular beats. ACh 1 in $10^{10}$ likewise may restore a normal beat. ACh 1 in $10^8$ to $10^7$ causes an increase in tonus and frequency, while ACh 1 in $10^6$ to $10^5$ causes an abnormally high frequency of beat followed by stoppage in systole. Atropine 1 in $10^5$ completely abolishes the ACh effect and in certain cases stops the heart in diastole. Adrenalin 1 in $10^6$ to $10^5$ also has an excitatory action on the heart of this species but this substance has not been extracted from the decapod heart as has ACh.

The number and arrangement of the ganglion cells and nerve fibers in the heart of Panulirus is similar to that in the majority of decapod crustaceans. Five large and four small cells have complex anatomical and physiological relations to one another and to the muscles, and some evidence regarding the manner in which they function has been obtained.

### 37. THE BLOOD PICTURE AND THE BLOOD SUGAR CHANGES DURING THE ALARM REACTION. Albert J. Dalton and Hans Selye, McGill University. (Lantern; 15 min.)

Previous experiments have shown that one of the cardinal features of the alarm reaction is the early appearance of leucocytosis. A more detailed study of this symptom has been made in conjunction with observations on changes in the erythrocyte count and blood sugar concentration. Muscular exercise and formaldehyde have been used as non-specific damaging agents in doses which previous experiments showed to be sufficiently large to elicit an alarm reaction.

Exercised rats show an early, marked lymphocytosis. This is followed by a short period of relative lymphopenia and this in turn is followed by a second, long lasting leucocytosis due to a great increase in polymorphonuclear leucocytes. Red cell counts in these rats show a preliminary rise followed by a more or less continuous fall to a low level 8 hours after the beginning of the experiment. The blood sugar curve has a similar form to that for the erythrocyte count.

A single subcutaneous injection of formaldehyde causes a preliminary leucopenia followed immediately by a marked and lasting leucocytosis, frequently in evidence 72 hours later. The blood sugar curve tends to follow that of the total white cell count in this case. The erythrocyte count shows first an increase followed by a decrease to below the normal level. This is followed by steady and long lasting increase to well above the normal level.

Although somewhat variable, the results obtained from studies on rabbits are in general similar to those above for rats. Further studies with various alarming agents on normal and adrenalectomized rats are now in progress.

38. TEMPERATURE ACTIVATION OF IMPURE AND CRYSTALLINE UREASE. Irwin W. Sizer, Massachusetts Institute of Technology. (Lantern; 15 min.)

The deamination of urea catalyzed by Jack bean urease was followed by determining colorimetrically after Nesslerization the amount of ammonia present at successive intervals during the course of the reaction. During the early part of digestion rate of nitrogen production was approximately constant. When log rate was plotted against 1/T the points fell along a straight line at temperatures below the inactivation temperature (40 to 65°C.). The slopes of the line correspond to activation energies ($\mu$ in the Arrhenius equation) of either 8700 or 11,700 calories per gram mol of enzyme, the value depending on the particular enzyme preparation which was used. With several preparations, however, a $\mu$ value of 11,700 was obtained below 21 to 26°C. and a value of 8700 above this critical temperature.

Results obtained on temperature activation of impure and crystalline urease were essentially the same. When the enzyme was dissolved in a medium lacking sulphite a $\mu = 8700$ was obtained. When the urease solution contained sulphite, however, $\mu$ was either 11,700 over the whole temperature range, or 11,700 below and 8700 above the critical temperature. The results are interpreted as suggesting that the urease molecule may have two centers or active groupings which are capable of activating urea. One ($\mu = 11{,}700$ is active only in the presence of reductants, the other ($\mu = 8700$) is active when the enzyme is oxidized or slightly reduced.

39. SOME ASPECTS OF SPIRALING IN CILIATES, TREES, AND VINES. W. E. Bullington, Randolph Macon College. (Lantern; 15 min.)

All free swimming ciliates swim in spirals; most vines and about 41% of trees grow in spirals. Most ciliates swim in one direction only, and may be classified into species on the basis of this direction. Most trees and vines grow in spirals in one direction only, and 67% of the species of trees may be classified on this basis into right spiraling trees and left spiraling trees.

Individuals in a few species of ciliates can swim in either right or left spirals, but none equally well. One direction is used in speed and distance swimming; the other in browsing and feeding, and this latter is always changed when the animal is greatly stimulated.

Individuals in a few species of trees can grow in either right or left spirals—right one year and left the next, and in extreme cases may change direction in the same trunk the same year. One vine has been found changing direction.

Left spiraling, nevertheless, predominates in all three—ciliates, trees and vines. In trees, this predominance is in the proportion of about 3 to 2.

### 40. EFFECT OF OSMOTIC PRESSURE ON THE MOTILITY AND VIABILITY OF FRESH-WATER PROTOZOA. John B. Loefer, Berea College. (Lantern; 10 min.)

Motility and viability limits were observed during experimental attempts to adapt five species of fresh-water protozoa to artificial sea water. Bacteria-free strains of Colpidium campylum, Glaucoma piriformis, Chlorogonium euchlorum, Euglena gracilis and Astasia sp. were used. The culture medium consisted of various dilutions of a Van't Hoff solution to which 0.2% Bacto-tryptone and 0.01% Difco Yeast extract had been added. They were successively transferred to increasingly concentrated salines. Time of exposure and growth in media ranging from 1, 5, 10, 15 . . . . 100% varied from 24 to 200 hours, pH was 6.7. Several series were carried through eleven to fifteen transfers. Astasia was never motile above a 40% Van't Hoff solution. Euglena developed no appreciable tolerance (40%). It withstood 35% on direct transfer. The viability of Chlorogonium increased from 40 to 65%. Glaucoma, at first viable from only 40% was finally viable from a 70% Van't Hoff medium. The acclimatization of Colpidium was greater; its tolerance increased from 30 to 95% Van't Hoff solution. The total salinity of the latter is higher than that of ordinary sea water. All organisms, except perhaps Astasia, developed their tolerance only gradually through a number of generations. The transfer from the concentrated solution back to the lower salinities could be made without fatal results. Decreased size and fewer contractile vacuole pulsations were observed in organisms from the media with relatively high salt content.

### 41. EFFECT OF THE ADDITION OF OLD CULTURE MEDIUM ON THE GROWTH OF COLPIDIUM CAMPYLUM. R. P. Hall and J. B. Loefer, New York University and Berea College. (Lantern; 8 min.)

Bacteria-free cultures of C. campylum were grown for several weeks in Erlenmeyer flasks in a synthetic medium containing 1% Bacto-tryptone. These aged cultures were filtered and the clear filtrate was added in the proportion of 1: 9 to similar freshly prepared medium. The mixture was measured into Pyrex culture tubes in 9 cc. amounts, and autoclaved along with the control tubes containing only the freshly prepared medium. Both series were subsequently inoculated from a young bacteria-free stock culture (initial count, 67 per cubic centimeter) and incubated in darkness at room temperature. Several tubes of the control and experimental series were fixed for counting at approximately 12-hour intervals. Counts made with a Whipple micrometer and Sedgwick-Rafter counting chamber indicate that growth in the experimental cultures was much better than in the controls. For example, after 84 hours the average number of ciliates in the cultures with old medium added was 1612 per cubic centimeter (an increase of 24 times) while the controls contained 435 (an increase of only 6.49 times). The differences were more pronounced after longer incubation periods, and in the last experimental cultures, fixed at 144 hours, the ciliates numbered 29,350 (an increase of 438 times), while controls had multiplied less than twenty times with 1327 organisms per cubic centimeter. Obviously, there was something in the old culture fluid which accelerated growth.

### 42. GROWTH OF ASTASIA SP. AND EUGLENA GRACILIS IN MEDIA CONTAINING INORGANIC NITROGEN. Henry W. Schoenborn, New York University. (Introduced by R. P. Hall.) (Lantern; 12 min.)

Six different inorganic media have failed to support growth of Astasia sp. Paired series were carried out in light and darkness in one of these media; in the early serial transfers, when an appreciable concentration of peptone was still present, growth was better in darkness than in light. Growth of Chlorogonium euchlorum in all six of these media showed them to be non-toxic. In testing Astasia sp. and Euglena gracilis for heteroautotrophic nutrition, four media were used (similar to above plus sodium acetate as an organic carbon source). Two of these have supported growth of Astasia sp. in darkness. In the seventh serial transfer, in which the calculated peptone concentration was reduced to $1.88 \times 10^{-10}$ gm. per cubic centimeter, slow growth occurred in one medium and questionable growth in the other. In the former medium macroscopic examination now shows growth in the eighth transfer. Preliminary observations indicate that iron may be one factor limiting growth in these media. Euglena gracilis has been grown in three of these four media; the results indicate that selection has occurred in each medium, as observed by Hall and Schoenborn in inorganic media.

### 43. THE BLOCKING OF EXCYSTMENT REACTIONS OF COLPODA DUODENARIA BY ABSENCE OF OXYGEN. Morden G. Brown, Stanford University. (Introduced by C. V. Taylor.) (Lantern; 10 min.)

The excystment process of the holotrichous ciliate protozoon Colpoda duodenaria results in a change in state from a metabolically inactive cell, the resting cyst, to an actively metabolizing cell, the trophic, free-swimming organism. In addition to this change in metabolic state, the excystment process involves a differentiation of cytoplasmic structures and escape from the cyst membranes. Though the excystment process thus may be very complex, it is perhaps exceptionally amenable to analysis since it may be started concurrently in a large number of uniform cells (same interfission age when encysted) by appropriate chemical changes in the environment; and further, the structural changes and escape from the membranes show the completion of certain physiological processes.

This analysis of the excystment processes depends chiefly on experimental data in which the time between addition of the excystment inducing solution and escape from the cyst membranes has been determined under controlled conditions of temperature, concentration of excystment solution, x-ray irradiation, and oxygen tension. The oxygen tension data show that the excystment time is unaffected by lowering the tension to 15 mm. of Hg, but that very low tensions block the excystment process at a point between the excystment period dependent on concentration of the excystment solution and the excystment period independent of the concentration of the excystment solution. Since no abnormalities appear for blocks of as long as 25 hours at 20°C. it is suggested that the enzyme systems that presumably become destructive in the unbalanced condition caused by absence of oxygen in the trophic stage are not activated until the later periods of excystment.

### 44. ENCYSTMENT IN THE CILIATE, WOODRUFFIA METABOLICA. Willis H. Johnson, Stanford University. (Lantern; 12 min.)

This large ciliate has been cultured for over 2 years with Paramecia as the only source of food. When grown in an inorganic balanced medium, Woodruffia forms resting or protective cysts only in the absence of food throughout a wide range of experimental conditions. Viable resting cysts were obtained throughout ranges a) of H-ion concentrations from pH 5.6 to pH 9.5, b) of temperatures from 10°C. to 37½°C. and c) of salt content of the medium from 0.012% to 1.2%, but only in the absence of food.

Crowding does not induce encystment with food present, but a certain amount of crowding shortens the encystment time in the absence of food. Metabolites are not effective in inducing encystment. A longer time is required for encystment in the excystment inducing solution used. There are indications that some nourishment is obtained from this solution which prolongs the free-swimming state. The use of starved Paramecia as food causes a gradual loss of the ability of Woodruffia to form cysts and ultimately leads to death.

This organism can encyst and excyst at least seven successive times without feeding, resulting in great reduction in size.

### 45. REGENERATION IN THE GENUS PARAMECIUM. Vance Tartar, Yale University. (Introduced by L. L. Woodruff.) (8 min.)

Excised parts are restored by paramecium with the same regularity as in other protozoa, regardless of the time during the division cycle at which the cells are cut. This result is offered as a correction of the previous conception that the ability of Paramecia to regenerate is lower than that of other protozoa, and that regenerative prowess varies with different races.

Fragments of P. bursaria containing neither the macronucleus nor the micronucleus are unable to regenerate, but exhibit a behavior department little from that of the normal nucleate cell. Cyclosis, positive thigmotactic reaction of the protozoon to bacterial masses, and coordination of the cilia are not under direct control by either of the nuclei.

Completely enucleate fragments of P. bursaria may survive for 1 to 6 days. The survival curves are statistically identical for a) fragments of green cells kept in daylight, b) fragments of green cells kept in the dark, and c) fragments of 'faded' cells kept in the dark. The unusual viability of enucleate fragments of P. bursaria, unique in this respect among seven species studied, is not therefore to be attributed to photosynthesis in the symbiotic green algae typical of this species.

Regeneration in paramecium is dissociable into two distinct processes: replacement of cell organelles and regeneration of shape. The latter is a growth process and occurs only when there is feeding and metabolism subsequent to cutting, while restoration of organelles (mouth, gullet, contractile vacuoles) is independent of food sources.

### 46. RACIAL DIFFERENCES WITHIN THE SPECIES CHAOS DIFFLUENS MÜLLER, THE COMMON LABORATORY AMEBA. A. A. Schaeffer, Temple University. (Lantern; Motion pictures; 15 min.)

Contrary to prevailing ideas, the common laboratory ameba, Chaos diffluens Müller, falls into several races or sub-species which maintain their characteristics for at least fifty generations, and apparently indefinitely.

Two such varieties, one with a smooth nucleus (the common one) and one with a folded nucleus, are found in the United States. The one with the folded nucleus is the only one that has been reported from Scotland. Apparently the Scottish variety is also the one more frequently encountered in the rest of Europe.

The American variety with a folded nucleus has apparently not been pedigreed. The Scotch variety has, however, been pedigreed in this country for about fifty generations under the same cultural conditions as the American variety with a smooth nucleus, and there can no longer be any doubt of a definite and permanent difference between the two varieties.

The literature, however, indicates that there may be more than two varieties. Stein reported a multinucleate ameba in 1867; Bütschli, two varieties of multinucleate amebas in 1876; Penard, a variety (nitida) with folded nuclei and 'embryos' in 1902; Schaeffer, two varieties, one with smooth and one with folded nuclei in 1916; Monica Taylor, one variety with folded nuclei, amitotic division and agamete production in 1923.

The chief differences between the common Scottish and the American varieties are: Scottish—folded nucleus, few ectoplasmic ridges, freely rolling moruloid (division stage), conspicuous division strands, very irregular mitosis; American—smooth nucleus, many ectoplasmic ridges, attached moruloid, rare occurrence of division strands, regular mitosis. No visible parasites have been discovered in either variety.

### 47. SOME PRODUCTS OF THE CELLULOSE DISSIMILATION BY THE TERMITE PROTOZOA. R. E. Hungate, University of Texas. (Introduced by J. T. Patterson.) (Lantern; 15 min.)

The wood-eating protozoa in termites are obligate anaerobes. They decompose cellulose and give off large quantities of metabolic products among which $CO_2$, $H_2$, and acetic acid have been identified. The quantity of products recovered indicates that all of the material digested is metabolized by the protozoa. The termite absorbs certain metabolic products (e.g., acetic acid) and metabolizes them aerobically to satisfy its energy requirements. If the termite does not remove the metabolic products of the protozoa the latter die.

Thus, in the symbiosis between the termite and the protozoa the following factors may be postulated: The termite ingests and comminutes the wood, provides anaerobic conditions, and removes the metabolic wastes which otherwise would kill the protozoa. The protozoa digest most of the cellulose and hemicellulose in the wood, utilize the digestion products in an anaerobic metabolism from which they derive energy for their life processes, and give off waste products, some of which are absorbed and oxidized to $CO_2$ and water by the termite, thereby satisfying the energy requirements of the latter.

The peculiar environmental requirements of the protozoa may explain their restricted distribution in nature.

48. LIFE CYCLE OF A SPOROZOAN PARASITE OF THE OYSTER. Herbert F. Prytherch, U. S. Bureau of Fisheries. (Lantern; 15 min.)

Since 1930 serious oyster mortalities have occurred in five different coastal regions from Mobjack Bay, Virginia to Lake Barre, Louisiana. In each instance an unusually weak condition of the adductor muscle and inability to maintain shell closure has been associated with a heavy concentration of spores in the muscle, gills and mantle. The mature spore containing a single vermiform sporozoite is the final developmental stage in the oyster.

Infected oysters are readily devoured by common mud crabs, Panopeus herbsti and Eurypanopeus depressus, in which hatching of spores takes place. The sporozoites develop rapidly into large cephaline gregarines which unite in pairs and migrate to the rectum of the crab where they become attached and form gametocysts. Rosettes of zygotes, or 'gymnospores' are released into the water with rupture of the gametocysts and enter the oyster gill by means of a pseudopod. They are generally picked up by phagocytes and transported in the circulatory system to nearly all parts of the host. In the blood cells the zygotes develop into sporozoites which after becoming surrounded by heavy, double walled sporocysts represent the characteristic resting stage found in Ostrea virginica.

This parasite is a heterogenetic gregarine with alternation of hosts, having vegetative and reproductive development in the intestine of decapod crustacea and sporogony in molluscs, particularly in lamellibranchs. The resistant, monozoic spores found in Ostrea are similar to those first described as Nematopsis by Schneider in 1892. The American form is a new species for which the name Nematopsis ostrearum is proposed.

49. HAPTOPHRYA VIRGINIENSIS NOV. SP., A PROTOZOAN PARASITE OF THE PICKEREL FROG, RANA PALUSTRIS LE CONTE. Samuel Lewis Meyer, Miller School of Biology, University of Virginia. (Introduced by L. L. Woodruff.) (Lantern; 7 min.)

Haptophrya virginiensis is a new species of astomatous ciliate found in the intestinal tract of the pickerel frog, Rana palustris Le Conte. The ciliate was discovered on July 7, 1938, while examining the contents of the rectum and posterior part of the small intestine of a pickerel frog obtained at Goode's Spring, about 0.7 mile south of the Mountain Lake Biological Station of the University of Virginia, Giles County, Virginia, at an elevation of approximately 4000 feet. The differences between the species previously placed in the genus Haptophrya Stein and the ciliate found at Mountain Lake are so significant that Haptophrya virginiensis is presented as a new species with the following diagnosis.

*Species diagnosis.* Haptophrya virginiensis new species. Body oblong, three to four times as long as broad, rounded and broadest anteriorly; posterior extremity truncate to rounded; anterior extremity provided with a sucking disc which flares anteriorly; pellicular striations absent; body cilia short and fine; cilia on the margin of the sucking disc longer and larger; contractile vacuole canalicular, straight to irregular sinuous, extending from the anterior to the posterior end of the body, communicating externally by a linear series of pores;

macronucleus elongate ovate to ellipsoidal, freely movable within the endoplasm. Length 300 to 400 $\mu$. A sexual reproduction by catenular budding.

The pickerel frog, Rana palustris Le Conte, is recorded as a new host for the genus Haptophrya Stein.

### 50. THE CORPUS LUTEUM OF REPTILES. Hermann Rahn, The Biological Laboratories of Cold Spring Harbor and Harvard University. (Introduced by A. B. Dawson.) (Lantern; 15 min.)

Post-ovulatory changes in the graffian follicles of seven species of viviparous and two species of oviparous snakes, the horned toad lizard and snapping turtle, reveal the formation of luteal tissue. In the viviparous snakes the corpora lutea may be recognized macroscopically as distinct yellow bodies throughout the whole period of gestation. The development of the luteal body in the viviparous snake Thamnophis and Potamophis seems to indicate that the granulosa layer is solely responsible for the formation of luteal cells, while the theca interna furnishes the supporting fibers and the blood vessels. The first change that occurs after ovulation is a great proliferation of the granulosa cells. These become very large and fill the shrunken cavity of the follicle. Then the infiltration of supporting fibers from the theca interna follows and finally the vascularization is completed. These vascularized bodies are maintained throughout gestation and degenerate slowly after parturition.

In the egg-laying snakes, Lampropeltis and Diadophis, and in the egg-laying species of horned toad lizard highly vascularized luteal tissue is likewise found in the recently ovulated follicles. Even in the snapping turtle luteal tissue is present which is invaded by supporting fibers but not blood vessels. In view of the relatively long time between ovulation and deposition of eggs in many of the oviparous forms and the definite formation of luteal tissue, it seems that in reptiles the corpus luteum is not necessarily associated with viviparity only, but also with the mere retention of eggs in the oviduct.

### 51. THE ROLE OF ESTROGENS AND PROGESTERONE IN THE INDUCTION OF MOUNTING ACTIVITY IN THE SPAYED FEMALE GUINEA PIG. William C. Young, John L. Boling and Brewster Rundlette, Brown University and Yale University. (Lantern; 15 min.)

Heat behavior in the female guinea pig consists of the copulatory response and usually male-like mounting activity. When both components of the sexual behavior pattern are displayed the temporal relationship is close, but no correlation exists between extent of mounting and length of heat. As far as induction of heat in this species is concerned, the role of estrogens and progesterone has been demonstrated, but the lack of any correlation between length of heat and extent of mounting has necessitated an independent investigation of this latter part of the sexual behavior complex.

Results from eighty-four injections have shown that mounting activity, like heat, is induced by estrogens in a small percentage of animals, but the synergistic action of small quantities of estrogens and progesterone is much more effective. When estrogen-injected animals were given progesterone 48 hours later (12 hours more than the latent period for estrogen-induced heat) 12.4% of the mounting activity occurred prior to progesterone injection, whereas 87.6% followed it. Furthermore, estrogen-conditioned animals did not mount in the presence of mounting estrogen-progesterone animals, although they did mount when subsequently injected with progesterone. Androstenedione, androstenediol, dehydroandrosterone and dehydroandrosterone acetate were not effective in the quantities used (0.25 mg.). The demonstration that estrogen-progesterone action is responsible for heat and mounting activity probably explains the close temporal relationship between the two.

The problem presented by the circumstance that no correlation exists between extent of mounting and length of heat is under investigation and will be discussed.

### 52. A DESCRIPTION OF THE 'HEAT RESPONSE' GIVEN AT BIRTH BY YOUNG MALE AND FEMALE GUINEA PIGS. John L. Boling, William C. Young, James G. Wilson and Richard J. Blandau, Brown University. (Introduced by A. D. Mead.) (10 min.)

A response consisting of straightening of the back and elevation of the pudenda which is identical with the heat response given by adult females, can be elicited from newborn males and females. The proportion of such individuals from which this response was obtained, the interval between the time of delivery and the time the response was first elicited, and the length of the period during which the response was obtained were ascertained for forty-six males and thirty-three females in twenty-eight litters.

One male and one female composing one litter and one male, the only animal born to another adult, did not give the response. In the remaining seventy-six young the first response was elicited immediately after birth or within 1½ hours. In forty-four males the response was obtained for an average of 2.4 hours, range 1.0 to 5.0 hours; in thirty-two females the response was obtained for an average of 2.8 hours, range 0.5 to 5.5 hours.

The observations suggest that at birth the guinea pig possesses an ability to respond to female hormones which is completely or largely lost in the normal maturing male. Since ovulation is usually associated with parturition, hormones produced in the developing follicles of the adult ovaries may be involved. The possibility is questioned, however, because the response was given by members of one litter born more than 2 days before the pre-ovulatory swelling normally associated with heat in the adult had begun.

### 53. DATA BEARING ON THE TIME REQUIRED FOR PARTURITION AND ON POSTPARTURITIONAL HEAT IN THE GUINEA PIG. John L. Boling, Richard J. Blandau, Arnold L. Soderwall, William R. Fish and William C. Young, Brown University. (Introduced by A. D. Mead.) (5 min.)

Data bearing on time required for parturition in twenty-seven animals are given.

In five animals one of which gave birth to a litter of three, three to litters of four, and one to a litter of five, less than 30 minutes were required. In three animals two of which gave birth to litters of two and one to a litter of three, less than 20 minutes were required. In fourteen animals one of which gave birth to a litter of one, three to litters of two, seven to litters of three, and three to litters of four, less than 15 minutes were required. In five animals two of which gave birth to litters of one, two to litters of two, and one to a litter of three, less than 10 minutes were required.

In thirteen instances the interval between birth of individuals was determined. This varied from 2 to 14 minutes, average 6 minutes.

For twenty-three animals data bearing on postparturitional heat were obtained. Five observed after parturition for 13, 22, 23 (two cases), and 48 hours, respectively, did not come into heat within this time. Ovulation occurred in four. In the fifth unruptured follicles were found similar to those in non-pregnant animals about the thirteenth day of the cycle. In eight animals heat began in less than 1 hour after parturition, in six 1 to 1½ hours later, in one 1½ to 2 hours later, in two 2½ to 3 hours later and in one 9½ hours later.

### 54. CYCLIC CHANGES IN THE FOLLICULAR APPARATUS OF THE OVARY OF THE ADULT RAT.

Charles E. Lane, University of Wichita. (Lantern; 15 min.)

Using a method to be described, the total volume of granulosa, antrum and theca in the ovary has been determined. Mitotic activity in the granulosa and theca has also been studied. Changes in volume of all components of the graafian follicle show definite correlations with the estrous cycle.

Mitotic activity in the granulosa and in the theca reaches its peak in metestrus. The total volume of granulosa is greatest during diestrus and the total volume of antrum attains its maximum in proestrus.

In the growth of the follicle the volume of the theca parallels and is but slightly less, quantitatively, than the volume of the granulosa. The total volume of the theca is greatest in diestrus and proestrus.

The ovary contains its maximum number of follicles during diestrus.

The significance of these data in the problem of the site of estrogen formation will be discussed.

### 55. THE CHANGES IN THE VAGINAL SMEARS AND ASSOCIATED CYCLIC PHENOMENA IN THE LOWLAND GORILLA (GORILLA GORILLA).

Charles R. Noback, Washington Square College, New York University. (Introduced by Harry A. Charipper.) (5 min.)

The first definite record of menarche ever to be observed in a lowland gorilla was found to occur at the beginning of the ninth year of age (at the New York Zoological Park). At that time, the presence of blood was noted on the vulva and verified as being of uterine origin. Following the date of menarche, nine menses and eight complete menstrual cycles were observed and correlated with changes in the vaginal smear contents and variations in the length of the urogenital cleft, the distance between the most dorsal point of the muco-cutaneous junction of the vulva and the dorsal surface of the clitoris at its base. This measurement proved to be the most consistent variable. The cycles averaged 43 days with a possibility of a 39-day cycle being the true average. The menstrual cycle was divided into the following five phases: menstrual, post-menstrual, copulation, ovulation and premenstrual. The menstrual phase showed the least variation in smear content and urogenital cleft measurement. Changes in linear dimension of the urogenital cleft were related to the different phases of the cycle, the gratest measurement occurring during the ovulation phase and smallest during the menstrual phase. The cyclic changes in the vaginal smears are similar to those reported for other primates including man. No correlation could be determined between rectal and vaginal temperature variations and the phases of the menstrual cycles observed.

### 56. Role of progesterone and other sex hormones in survival of pseudopregnant, adrenalectomized ferrets. Robert Gaunt and H. W. Hays, Washington Square College, N. Y. University and Princeton University. (Lantern; 10 min.)

The authors have shown previously that the pseudopregnant ferret, like the dog, withstands adrenalectomy until near the end of the pseudopregnant period. Estrus on the contrary accelerates the appearance of adrenal insufficiency, and the cortical hormone requirements of ferrets in estrus are approximately doubled. The same phenomenon is noticed when estrus is induced by estrogenic hormones or gonadotropic agents. The cause of the toxicity of the estrogens is not clear.

The secretion of progesterone alone probably gives an adequate explanation of the life extension during pseudopregnancy. Injections of crystalline progesterone in minimal doses approximating 2 mg. per day sustained life and excellent health throughout a treatment period of 20 to 32 days in six adrenalectomized ferrets, of which three were females, two castrate females and one a castrate male. In doses of 5 mg. per day it relieved an established adrenal insufficiency (four cases).

Testosterone propionate in doses of 5 to 10 mg. per day was non-toxic and non-beneficial to adrenalectomized ferrets.

### 57. Cortical hormone-like action of progesterone in rats. Robert Gaunt, Warren O. Nelson and Eleanor Loomis, Biological Laboratory, Cold Spring Harbor; Department of Biology, Washington Square College, N. Y. University; and Department of Anatomy, Wayne University. (Lantern; 5 min.)

Gaunt and Hays found progesterone to be an effective cortical hormone substitute in adrenalectomized ferrets (above abstract). We have noted a similar, but weaker, action of progesterone in rats. One to 2 mg. progesterone per day extended the life span of 30-day-old adrenalectomized rats beyond that of untreated littermate controls. Twenty-two untreated controls died at an average of 7.5 days. Of nineteen treated animals, ten died during treatment at an average of 10.4 days and nine survived during treatment. Weight gains in treated animals were four times those of untreated controls. Failure to maintain life in all cases was probably due to inadequate dosage. On the basis of equivalent body weight it took ten or more times as much progesterone to maintain adrenalectomized rats as ferrets. The need for such large dosage in this species probably accounts for the several previous failures to notice these cortical hormone-like effects of progesterone.

Progesterone (7.5 mg.) failed, however, to act like cortical extract in protecting adrenalectomized rats against 'water intoxication' symptoms following administration of excess quantities of water. Estrone and testosterone propionate were likewise without effect, despite their known action on electrolyte metabolism of intact animals. The failure of progesterone to act in acute tests of this type indicates some difference between the rate or type of its action and that of cortical hormone.

### 58. Tests indicating absence of progesterone in fowl ovary. Oscar Riddle and James P. Schooley. Carnegie Institution. (Lantern; 10 min.)

Utilizing the recently introduced technique of McGinty and associates for demonstrating qualitatively the presence of extremely minute amounts of progesterone we have attempted to learn whether the corpus luteum hormone, progesterone, is produced in birds. Two types of bird ovaries were taken as possible sources and extracted according to the method of W. M. Allen; in each case approximately 15 gm. of fowl ovaries (yolk previously removed from follicles) were extracted. One sample of ovarian tissue was obtained from actively laying hens; the other sample, of similar fresh tissue, was from hens in various phases of egg production but previously injected with a pregnancy urine preparation (prolan) daily for 3 days in an attempt to luteinize their ovaries. Neither of the resulting purified extracts contain enough (1 $\gamma$) progesterone to produce a positive reaction in the prepared uterus of a suitable rabbit. The best available evidence on this point therefore indicates that such fowl ovaries either do not produce progesterone or that they produce an amount nearly or quite insignificant in comparison with that formed in the ovaries of adult mammals. Attention is drawn to the evolutionary aspect of this result.

### 59. Effects of estrogen and estrogen plus androgens upon the testes and gonaducts of adult male pigeons. Carroll A. Pfeiffer and W. U. Gardner, Yale University. (Introduced by Edgar Allen.) (Lantern; 15 min.)

The testes of young adult male pigeons are suppressed and any rudiments of the mullerian duct which may persist (five out of fourteen cases) are stimulated by injection of 1000 i.u. estrin daily. The testes shrink to one-tenth their original weight in 22 days. Active spermatogenesis ceases, and only spermatogonia and a few spermatocytes remain. Up to 70 days, the duration of this experiment, the testes remained in this quiescent condition, resembling in every detail the inactive testis of seasonal breeding birds, indicating suppression of the pituitary.

When male hormone (testosterone propionate, 1.25 or 2.5 mg. daily) is also injected, the testes do not regress as described above but stimulation of the mullerian rudiments is not interfered with (four out of seven cases). The testes maintain their normal volume. Spermatogenesis continues normally, in some cases for 43 days or more. When the smaller amount of male hormone is administered or injection is continued for a longer time, spermatogenesis is damaged to some extent but was not completely destroyed up to 56 days (termination of experiment). Under both conditions the portion of the mullerian duct corresponding to the cephalad part of the normal female oviduct responded although a middle portion occasionally persisted. In two cases a middle portion was found on the right side. Histologically these ducts resemble the normal oviduct except the portion distended with secretion due to the blind ending. No macroscopical rudiments were observed in eleven control males.

### 60. Effect of testosterone propionate on the black-crowned night heron.

G. K. Noble and M. Wurm, American Museum of Natural History. (Lantern; 12 min.)

Both sexes of the night heron (Nysticorax n. hoactli) develop during the breeding season, a marked melaninization of the lores, lower mandible and buccal cavity, also a vascularization of the legs which gives them a pinkish color. These modifications may be produced in gonadectomized adults or in immatures by injections of tesosterone propionate (Ciba). Castration during the breeding season effects a loss of pigmentation in male and female. Estrogens (theelin, oestrone acetate, amniotin and follutein) fail to produce any of these changes. Hence the seasonal changes in the secondary sexual characters of the night heron are probably due to male hormone in both sexes.

Estrogens hypertrophy the oviduct of the spayed adult or the immature but have no effect on the vasa defferentia. Testosterone propionate hypertrophies the latter but also causes some enlargement of the oviduct.

Testosterone propionate will induce male sexual behavior in adult females or in immatures of both sexes. Even in month-old chicks it will make the voice more gutteral and will induce territory defense, nest building, all male courtship ceremonies, copulation and later brooding. Differences between the sexual behavior of the adults seem regulated only by differences in the amounts of male hormone normally found in these birds. Estrogens alone fail to stimulate any breeding behavior in either sex.

### 61. Influence of injected male hormone on the social hierarchy in small flocks of hens.

W. C. Allee and Nicholas Collias, The University of Chicago. (Lantern; 15 min.)

Hens have a definite and relatively stable social order which is revealed by the number of flock mates each pecks with impunity. To explore the underlying physiology of this order ¼ to 1¼ mg. of testosterone propionate was daily injected intramuscularly into mature White Leghorn hens which had been under daily observation for over 6 months. Injections continued 51 and 54 days. Other flock members were injected daily with neutral sesame oil and the hormone was assayed by the standard capon-comb test.

June 6th, RG ranked lowest in her flock of eight; August 1st, after receiving 1 mg. daily (3 mg. for the last 5 days), RG pecked all except BR which had received ½ mg. injections; BR had successfully revolted against two superiors. June 6th, GG (½ mg. injections) outranked only BG (¼ mg.) in her flock of seven; August 1st, GG was the alpha bird and BG now pecked two former superiors.

August 17th, BY (1¼ mg.), RY (1 mg.) and BG (¾ mg.) ranked lowest among nine pen mates; October 20th, BY had climbed from lowest to highest position; RY pecked four; BG, ill and removed from experimentation, had won one reversal. Illness excepted, no other reversals occurred in these flocks. As is customary with hens, social positions of injected birds, once won, were retained.

Comb growth started immediately with injections; laying usually stopped; after a time four injected hens crowed at least once; all ceased crowing soon after injections stopped.

### 62. EFFECTS OF HYPOPHYSECTOMY AND ADRENALECTOMY ON RENAL ACTIVITY IN THE RAT. E. L. Corey, University of Virginia Medical School. (Lantern; 10 min.)

The experiments reported represent over 200 individual fluid metabolism determinations. The experimental period was limited to 24 hours with water supplied ad lib. All animals were fasted during the experiments. Urine was collected under toluene in graduated cylinders and urinary chloride determined by the Volhard titration method.

Hypophysectomy resulted in an immediate polyuria and polydipsia (the latter due to excessive fluid losses through the kidneys) which subsided after about 4 to 6 days. Following simultaneous removal of pituitary and adrenal glands no such effects were observed. This was interpreted as evidence of an antagonistic action on fluid metabolism of the posterior lobe of the pituitary and the adrenal cortex.

In all cases, hypophysectomy, hypophyso-adrenalectomy or adrenalectomy resulted in a dilution of urine chlorides during the first 24 hours after operation with subsequent concentration within a few days to slightly above the normal level. No such chloride dilution was observed following 'sham' hypophysectomy, splenectomy or thyroidectomy. The injection of cortico-adrenal extract into hypophysectomized rats was followed by chloride dilution, as in hypophysectomy alone.

The similar results (as regards chloride dilution in the urine) which were seen to follow hypophysectomy, hypophyso-andrenalectomy or adrenalectomy, were interpreted as indicative of hypofunction of the posterior lobe of the pituitary immediately following adrenal removal.

### 63. INTRA-OCULAR HOMOTRANSPLANTATION OF SUPRARENAL GLANDS IN THE RAT. C. Donnell Turner, Northwestern University. (Lantern; 10 min.)

These experiments were begun in order to analyze the fáctors which regulate the incorporation and persistence of suprarenal transplants. Whole glands from young donors, ranging in age from birth to 32 days, were transplanted unilaterally and bilaterally into the eyes of normal and suprarenalectomized adult hosts. A total of 387 glands were introduced into the anterior chambers of 245 hosts. The grafts were recovered at intervals ranging from 12 hours to 5 months after insertion and studied histologically and cytologically.

Among normal hosts, a total of 51% of the bilaterally transplanted glands incorporated and contained cortical tissue at the time of removal; 64% of the unilaterally transplanted glands persisted under the same conditions. The extent of cortical regeneration and the frequency of persistence were augmented by suprarenalectomy and by homopituitary implantation. The evidence seems to indicate that the corticoadrenotropic principle of the anterior hypophysis is one of the factors which regulated cortical differentiation. Medullary tissue seldom persisted in whole transplants.

Within 24 hours subsequent to the introduction of whole glands into the anterior chambers the central parts of the transplants usually degenerated, leaving only the capsule and a peripheral layer of glomerulosa. The new cortex was restored by proliferation from the glomerulosa or by the differentiation of unspecialized cells in the capsule. Cytological studies on normal and transplanted glands indicated that the cortices are composed of one type of secretory cell present in various stages of differentiation and post-secretory degeneration.

### 64. THE RELATION BETWEEN THE AGE OF THE CULTURES FROM WHICH CHILOMONAS IS TAKEN AND THE RATE OF REPRODUCTION IN FRESH CULTURE FLUID. S. O. Mast and D. M. Pace, Johns Hopkins University. (Lantern; 15 min.)

Chilomonads from young, medium-age and old cultures were washed and put into fresh culture fluid in depressions on slides, one in 0.025 cc. in each depression, thirty-six depressions for each culture. Those from each culture were divided into three groups (A, B and C), and examined hourly. In A and B one chilomonad was removed from each depression after each division; in C none. In A the one removed was discarded; in B it was put into 0.025 cc. fresh culture fluid and the one left discarded.

In all three sets it required for the first division 11± hours for the chilomonads from the old culture; 7.5± hours for those from the medium-age culture and 6+ hours for those from the young culture. In all the rate of reproduction increased to a maximum of one division in 4+ hours and then decreased but the maximum was reached much sooner in C than in A and B. In A the decrease in rate of reproduction continued for about 3 hours and then became nearly constant and the same for the chilomonads from all three cultures. In B and C it decreased indefinitely but much more rapidly in C than in B.

These results indicate that Chilomonas produces a substance which in low concentration accelerates growth and in high concentration retards it; that the concentration of this substance in the chilomonads varies directly with the age of the culture and that it diffuses out slowly after they are transferred to fresh culture fluid.

### 65. THE ADJUSTMENT OF THE MARINE AMOEBA, FLABELLULA MIRA SCHAEFFER, TO CHANGES IN THE TOTAL SALT CONCENTRATION OF THE EXTERNAL MEDIUM. Dwight L. Hopkins, Mundelein College. (Lantern; 15 min.)

Flabellula mira can withstand extensive and rapid changes in the total salt concentration of its environment. It can be transferred directly from sea water to 5% sea water (sea water diluted twenty times with distilled water) or from 5% to 100% sea water and yet within a period of a few hours adjust itself, feed, grow and reproduce normally.

When transferred from 100% to 5% sea water the amoebae immediately swell to several times their original size then gradually lose volume until the original volume of 100% sea water is regained. This loss of volume is not due to the action of cytoplasmic vacuoles emptying to the outside, but instead to diffusion of water and other substances from the amoebae. When amoebae living in 5% sea water are transferred to 100%, they at once shrink greatly and then within a few hours, due to inward diffusion of water and salts, the original volume is regained.

When the amoebae have become adjusted to a given concentration of salts and have begun to feed actively, fluid material is eliminated from the cell by contraction of vacuoles to the outside. The rate of elimination of this fluid from amoebae adjusted to a medium is directly proportional to the volume of the amoebae and inversely proportional to the total concentration of salts in the outside medium.

66. THE FUNCTIONS OF CONTRACTILE VACUOLES. J. A. Kitching, University of Bristol, England. (Introduced by E. Newton Harvey.) (Lantern; 15 min.)

The contractile vacuoles of Protozoa are believed to act as osmoregulatory organs, baling out water as fast as this comes in through the body surface by osmosis. Such a process would involve an active separation of solutes from water at the vacuolar membrane. The rate of output of fluid by the contractile vacuoles of various fresh water Protozoa is decreased by an increase in the osmotic pressure of the external medium; conversely that of various marine Protozoa is increased by an decrease in the external concentration. The contractile vacuoles of peritrich Protozoa are almost completely inhibited by dilute cyanide, and under suitable circumstances a large increase in body volume results. On a return of the organism to a cyanide-free medium the contractile vacuole recovers and operates at first with an unusually high rate of output, and the body gradually shrinks.

There is no direct chemical evidence to show that contractile vacuoles excrete nitrogenous waste. The part played by neighboring granules remains unknown. The question of excretion must therefore be left open. Only a small proportion of the water evacuated by the contractile vacuoles of fresh water Protozoa is contributed by food vacuoles, but in some marine Protozoa food vacuoles are probably the chief source of the water evacuated. It is possible that the contractile vacuole may perform several functions in the same organism.

67. THE SPERM-ASTER AND PROTOPLASMIC MOVEMENT. Robert Chambers, New York University, Washington Square College. (Lantern; 15 min.)

It is known that the diminutive aster, which appears about the sperm-head a few minutes after insemination in the Echinoderm egg, rapidly grows until it occupies the bulk of the egg and its center coincides with the egg's center.

It has also been indicated that the main mass of the astral radiations is a gel, hence the development of the aster represents a reversible gelation of the egg cytoplasm which was previously in the sol state.

Eggs of Arbacia punctulata were compressed in sea water several minutes after insemination causing the fertilization membrane to rupture and the formation of small exovates only about 4% of the egg volume.

In one case a small exovate was formed which happened to contain the diminutive sperm-aster, 8 minutes after insemination. With the progressive enlargement of the sperm-aster the exovate increased in size at the expense of that part of the egg within the fertilization membrane. The existence of a flow was detected by the steady migration of cytoplasmic granules through the protoplasmic stalk joining the two parts of the dumb-bell shaped egg. The egg nucleus also was seen to move with the stream out into the exovate and finally come to rest within the central astral lake.

Eventually the entire egg lay outside its fertilization membrane having become incorporated in the exovate.

Other exovates which did not contain the sperm-aster were drawn back into the fertilization membrane.

This constitutes critical evidence that the aster causes a specific redistribution and flow of the cytoplasm to center about itself.

68. STRUCTURE OF THE INTRANUCLEAR CHROMOSOME. William R. Duryee, New York University, Washington Square College. (Lantern; motion picture; 15 min.)

It has recently been shown that a cell nucleus can reversibly be made completely transparent without loss of the identity of the normal chromosome number. In the germinal vesicle of the salamander, Triturus pyrrhogaster, interkinetic chromosomes constitute the largest yet observed (two pair over 500 $\mu$).

By micromanipulative technique the finer physical structure of each part of a chromosome: chromonema cylinder, chromomere granule, chromomere loop, and adherent developing nucleoli, can all be established. Each transverse chromomere loop has a relatively greater tensile strength than that of the longitudinal chromonema and is itself composed of a translucent elastic cylinder containing denser segments.

A nucleolus has also a solid gel structure which can be broken open with microneedles, but this gel can be reversibly shifted toward the sol state by increasing the potassium-calcium ratio. During the process viscous nucleolini are exuded in the form of drops or pseudochromosome strands or the entire nucleolus may become dissolved.

Shedding of nucleoli and sloughing off of chromomere loops are regarded as part of normal nuclear function in supplying materials to the cytoplasm. Consequently intranuclear 'expanded' chromosomes should be regarded as in a physiologically active phase compared with their 'contracted' condition during mitosis.

69. THE EFFECT OF CHANGES OF URINE pH ON THE ELIMINATION OF NEUTRAL RED AND PHENOL RED BY THE PERFUSED FROG KIDNEY. Rudolf T. Kempton, Vassar College. (Lantern; 15 min.)

In a recent paper on perfusion experiments of the frog's kidney, indications were presented that the elimination of neutral red, a salt of a basic dye, can be explained as a physical diffusion depending upon a pH gradient. From this point of view a study has been made of the excretion by the perfused kidney of phenol red, a salt of a sulphonated acid. In this case the dye output by the kidney is independent of changes of the pH of the urine, indicating that the mechanism involved is different from that concerned with neutral red.

70. OXYGEN CONSUMPTION AND METHYLENE BLUE REDUCTION IN RELATION TO VERONAL INHIBITION OF LUMINESCENCE. Frank H. Johnson and Edward L. Chambers, Princeton University. (Lantern; 10 min.)

The luminescence of marine luminous bacteria (Achromobacter Fischeri) suspended in isotonic sodium chloride solution containing peptone and glycerin, together with phosphate buffer at a pH of 8.2, is readily inhibited by veronal (sodium diethylbarbituric acid). Small concentrations reduce luminescence intensity with no appreciable effect on total oxygen consumption. Luminescence intensity is reduced 50% in 0.025 M veronal with only a slight reduction in rate of oxygen uptake. For any given veronal concentration causing an appreciable inhibition of respiration, the per cent inhibition in rate of oxygen consumption is the same as the per cent inhibition in rate of methylene blue reduction by portions of the same suspension within the limits of experimental error. Under the experimental conditions employed, the activity of the dehydrogenases is proportional to, and evidently determines, the rate of total oxygen consumption. The sensitivity of luminescence to veronal suggests a more specific blocking of the light producing reaction.

The action of veronal in moderate concentrations is completely reversible, but in strong concentrations only partially so.

### 71. THE BACTERICIDAL ACTION OF HIGH AND LOW INTENSITIES OF ULTRAVIOLET RADIATION. H. C. Rentschler and Rudolf Nagy, Research Laboratory of the Lamp Division, Westinghouse Electric and Manufacturing Co. (Introduced by W. C. Curtis.) (Lantern; 15 min.)

Coli bacteria were uniformly seeded on Petri plates and exposed to measured amounts of ultraviolet radiation. For the same amount of radiation (intensity × time) the same percentage of colonies were killed when the radiation was applied by a single flash discharge of a condenser requiring approximately $10^{-6}$ seconds as with a low intensity transformer discharge requiring minutes to produce the same amount of radiation. Thus, over this range of intensities the reciprocity law for the killing of coli bacteria by ultraviolet radiation holds.

Lower intensities requiring hours to produce the same amount of radiation were found to be several times as effective as the higher intensities. Thus, radiation which produces a 50 to 60% reduction of colonies with an intensity requiring a 10-minute exposure results in a 99.9% killing when applied over a period of a number of hours (10 to 30 depending upon the temperature). For still lower intensities where the bacteria multiply more rapidly than they are killed, the reduction in colonies on the plates is again reduced, and for sufficiently low intensities becomes negligible.

Other tests indicating the cause for these results will be described and the importance of the results will be discussed.

### 72. THE EFFECT OF POTASSIUM AND OF UREA ON THE BREAKDOWN OF THE PIGMENT GRANULES IN THE FERTILIZED EGG OF ARBACIA. Leon Churney, University of Pennsylvania. (Introduced by L. V. Heilbrunn.) (Lantern; motion picture; 15 min.)

The red pigment granules in the cortex of fertilized sea urchin eggs behave differently from the same granules in the egg before fertilization. They disintegrate when eggs are immersed in isotonic KCl or in isotonic urea solutions 10 minutes after fertilization, although neither of these reagents is effective before this time. $NH_4Cl$ acts like KCl; whereas the chlorides of Na, Ca and Mg are ineffective.

The mechanism of breakdown in KCl and $NH_4Cl$ solutions differs from that in urea. Granule breakdown in KCl or $NH_4Cl$ solutions is apparently dependent on Ca release, but urea acts on the granules directly. Thus, whereas in urea solutions all the granules in the egg explode instantaneously; in KCl or $NH_4Cl$ solutions only a few disintegrate upon immersion. Also, granules isolated from eggs do not disintegrate in KCl or $NH_4Cl$, but they are unstable in urea solutions.

The minimal concentration of urea effective on the isolated granules is approximately 0.30 M. This is also the minimal concentration effective on the granules in the living cell and there is thus additional evidence favoring the view that within the cell urea acts on the granules directly. If fertilized eggs are given two preliminary washings in an isotonic sodium citrate solution before and during the period of susceptibility (in order to remove Ca from the cell cortex), then following this treatment K and $NH_4$ are incapable of causing granule breakdown. The effectivity of urea, however, remains unimpaired.

### 73. THE RELATION OF THE CALCIUM ION TO THE IRRITABILITY OF ISOLATED MUSCLE FIBERS. L. V. Heilbrunn and E. W. Ashkenaz, University of Pennsylvania. (Lantern; 15 min.)

In earlier work from this laboratory, it was shown that the effect of stimulating agents on the protoplasm of ameba and marine eggs may to a large extent be prevented if the calcium is removed from the cell cortex by previous immersion in oxalate or citrate solutions. Similar experiments have now been performed on isolated frog muscle fibers. If these are immersed in solutions of sodium, potassium or magnesium salts in the absence of calcium, they become insensitive to ordinary electrical shocks (condenser or induction coil). However, in the absence of calcium, the fibers lose none of their sensitivity to ultraviolet radiation, and they are also sensitive to faradic stimulation. It seems probable that ordinary electric stimulation affects only the outer surface of the muscle fiber, whereas ultraviolet radiation penetrates more deeply. Perhaps in a muscle fiber the outer cortex is not the only sensitive region. Indeed, as might be expected, the indications are that the single muscle fiber is a more complex system than an ameba or an egg cell.

If isolated muscle fibers are placed in isotonic solutions of calcium salts, they shorten rapidly. This shortening is due primarily to an effect on the injured ends of the single fiber preparation, for it is only when the ends are immersed in the solution that shortening occurs.

These facts are considered in the light of the calcium release theory of stimulation.

### 74. THE INFLUENZE OF TEMPERATURE UPON THE DYNAMIC CONSTANTS OF MUSCLE. Herbert Shapiro,[1] University College, London and Clark University. (Lantern; 10 min.)

As has been shown recently by A. V. Hill from thermal studies, the dynamic behavior of a muscle during contraction may be summarized by the following formulation:

$$(P + a)\ (V + b) = b\ (P_0 + a)$$

where P represents the steady force the muscle exerts during shortening at velocity V, $P_0$ the maximum isometric tension, $a$ and $b$ are constants. The constants may be determined for any given muscle independently of heat measurements. By means of the Levin-Wyman ergometer, the steady tension exerted at predetermined rates of shortening may be read off from the indicator diagrams.

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[1] Fellow of the John Simon Guggenheim Memorial Foundation.

From such data the effect of temperature upon the constants may be calculated. Typical results, for determinations at four different temperatures upon the same muscle (sartorius of Hungarian bullfrog, R. esculenta) are

| *Temperature (°C.)* | $a/P_0$ | *b (% resting length)* |
|---|---|---|
| 0.0 | 0.230 | 39 |
| 2.7 | 0.284 | 67 |
| 14.2 | 0.175 | 174 |
| 24.0 | 0.200 | 256 |

$a/P_0$ is relatively unaffected by temperature; b has a large temperature coefficient (activation energy of approximately 12,000 calories). From these results the inference is drawn that in muscle the form of the isometric contraction is determined by the rate at which energy is fed from the chemical system to the contractile mechanism, and that temperature exerts its usual effect upon the kinetics of the chemical system. Detailed studies have also been made of the effects of $CO_2$, and of the values of the constants during stretches.

75. THE BEHAVIOR OF THE CROSS STRIAE OF MUSCLE FIBERS AND THE SIGNIFICANCE FOR CERTAIN PROBLEMS OF MUSCLE ORGANIZATION. Carl Caskey Speidel, University of Virginia. (Lantern; 12 min.)

The number of cross striae in a muscle fiber is definite. This number does not change during contraction, relaxation, stretching, or retraction. Shortening of a fiber is accompanied by shortening of individual sarcomeres, not by multiplication of sarcomeres. These data do not support the theory of muscle contraction recently proposed by Carey.

Contraction bands replace resting cross striae only when the contracting sarcomeres shorten greatly. They do not replace resting cross striae in contracting fibers in which the sarcomeres shorten slightly.

In a normal muscle fiber the cross striated substance and the unstriated sarcoplasm are in the form of a gel. Following irritation or injury, however, the sarcoplasm may change to a sol state while the cross striated substance remains a gel. Kühne's widely publicized observation of a nematode crawling through an injured muscle fiber has been wrongly interpreted as indicating that the cross striae are fluid.

The helicoid theory of cross striation is not upheld. Interference phenomena, combined with differential tension adjustments within the muscle fiber, seem sufficient to account for the variable complex patterns of cross striation.

Fibers within the living animal in a state of neurogenic tonus exhibit no discernible histological differences from fibers which are not in neurogenic tonus. (See also demonstration no. 120.)

76. TIME-TEMPERATURE RELATIONS IN WHITEFISH INCUBATION. John W. Price, Ohio State University. (Lantern; 12 min.)

White fish eggs incubated in aerated lake water at controlled temperatures of 0°, 0.5°, 2°, 4°, 6°, 8°, 10°, and 12°C., failed to hatch at either 0° or 12°C. At 10°C. 1.5% hatched, 65% hatched at 0.5°C., and an intermediate proportion hatched at intermediate temperatures.

Percentage of abnormal embryos which developed varied directly with temperature between 2° and 12°, all embryos being abnormal at 12°C.; but none at either 0.5°, or 2°C. At 0°C., however, 10% were abnormal. Normal development occurred from 0.5° to 6°C., with an optimum temperature of 2°C.

Total incubation time ranged from 29.6 days at 10°C.; to 141 days at 0.5°C. The time (t) required to attain a certain stage of development is expressed in equations

$$t = m \cdot a^{-T\ 0^\circ - 6^\circ}; \qquad t = m \cdot a^{-T\ 6^\circ - 12^\circ}$$

where temperature, T, is a negative exponent of the constant, *a*, whose value differs above or below 6°C., a critical temperature. Values of *a* above 6° fluctuate about 1.12; those of *a* below 6° fluctuate about 1.19 as a mean. Applying Arrhenius' equation $\mu$ values for various stages range around 20,000 above 6° and 30,000 below 6°C. Semi-log curves expressing the above relations form series of straight lines essentially parallel to one another, except for the stage just prior to hatching.

Deviations in $\mu$ values at hatching, indicate the probability of the introduction at that time of factors peculiar to that process.

### 77. The production of robin pigment in White Leghorn feather cells by skin ectoderm grafts of the robin embryo. Mary E. Rawles, University of Rochester. (Lantern; 15 min.)

Small pieces (0.6 × 0.3 mm.) of skin ectoderm, stripped from the posterior head region of robin embryos of developmental stages equivalent to 90- to 96-hour chicks, were grafted (as free as possible of underlying mesenchyme) to the wing bud base of 72 hours White Leghorn hosts. At hatching the hosts showed areas of colored down varying in intensity from blackish brown to pale cinnamon, covering most of the wing and contiguous regions. In one case which attained sexual maturity, this down was replaced by juvenile contour feathers of mosaic coloration—the distal portion reddish brown, the proximal slate gray—as in the breast feathers of the adult robin, and of identical color. In addition white basal areas (host-colored) appeared on the remiges and larger coverts. After moulting the entire graft region became host-colored as in similar grafts between different breeds of fowl.

A microscopic study of the proximal and distal barbules of the inner and outer vanes, and of the typical downy barbules of the robin-colored contour feathers of the graft area, show unmistakably that the feathers are of Leghorn structure. The shape and size of the pigment particles (easily seen in down cells) are definitely robin but their distribution is like that of the common breeds of pigmented fowls.

These data support the already proposed hypothesis that in grafts of this type the feather germs are of host origin, but the coloration is produced by chromatophores from the implanted donor tissue which migrate into the epidermal 'collar' of the host feather germ.

### 78. MOSAIC DEVELOPMENT AND REGENERATION OF THE EYE-FORMING AREA IN THE CHICK EMBRYO. Nelson T. Spratt, Jr., University of Rochester. (Introduced by B. H. Willier.) (Lantern; 15 min.)

The eye-forming area of definitive primitive streak and head-process stages, as delimited by the method of chorio-allantoic grafting, together with the larger part of the area pellucida anterior to it has been cultured in vitro. When grown separately, right and left halves of this area produce, respectively, the right and left halves of the forebrain, each with an optic vesicle. Usually the dorsal and ventral brain walls contralateral to the optic vesicle fail to heal together. Anterior and posterior halves of the area pellucida separated by a transverse cut passing through the center of the eye-forming area produce, respectively, the anterior and posterior halves of the forebrain. Development following these cuts and variations in them shows that the forebrain-eye-forming area at the definitive primitive streak stage is a mosaic so far as morphogenesis is concerned.

After complete removal of the forebrain-eye-forming area of definitive primitive streak and head-process stages, the blastodisc compensates for the loss by regenerating either a complete forebrain with optic vesicles (28 out of 193 cases) or a less completely formed and distorted forebrain (111 out of 193 cases). This process is still less complete, or usually absent, in head-fold and later stages. Experiments on stages earlier than the definitive primitive streak indicate a marked increase in the capacity to reform the excised part (twenty-seven out of thirty cases). In general, the degree of regeneration obtained seems to depend upon the size of the piece removed and the stage of the embryo used.

### 79. THE EFFECT OF HOMOTYPICALLY CONDITIONED WATER AND THE SIZE OF THE CONTAINER ON THE GROWTH OF AMBLYSTOMA LARVAE. Cranford Hutchinson, The Morris Biological Farm of The Wistar Institute. (Introduced by Helen Dean King.) (Lantern; 10 min.)

Experiments testing the effect of homotypically conditioned water on the growth of urodele larvae show results similar to those obtained by Allee and associates on fish. Growth was compared between groups kept in similar finger bowls in which water was 1) left unchanged, 2) changed daily with fresh homotypically conditioned water and 3) changed daily with fresh spring water. Unchanged water appeared as favorable for growth as freshly conditioned water during the first 100 days of the experiment but after that time the unchanged water caused slower growth. Fresh spring water was definitely unfavorable for growth and caused some mortality during the course of the experiment (120 days). Similar groups raised in aquaria grew faster than those kept in finger bowls though the volume of water per animal was the same. These experiments corroborate the findings of Allee and associates and show that the same phenomenon which they have found with fish also applies to Amphibia.

### 80. FURTHER EXPERIMENTS UPON AXIAL POLARIZATION IN THE EMBRYONIC LIMB RUDIMENT OF THE SALAMANDER. F. H. Swett, Duke University School of Medicine. (Lantern; 10 min.)

1. Fore limb rudiments of Amblystoma punctatum (Linn.) have been shown not to undergo reversal of the prospective polarity of their dorsoventral axes when transplanted, in inverted orientation, to the lateral surface of the myotomes. The same type of experiment carried out upon A. tigrinum, and reciprocal heteroplastic grafts between these two species have given similar results.
2. Limb-forming tissue has been transplanted from embryos of various early stages to the myotome site in embryos of tail bud stages. The positive cases have shown the establishment of prospective polarity in the dorsoventral limb-axis to occur as early as the gastrula stage of development.
3. Limb rudiments grafted from tail bud stages to the flank of older embryos have provided evidence that the flank tissue may retain its polarity-reversing effect upon the inverted dorsoventral limb-axis at least within the age limits (up to stage 41) imposed in these experiments. In this respect the flank tissues differ markedly from those of the head.

### 81. SEX DIFFERENTIATION IN TRIPLOID SALAMANDERS (TRITURUS VIRIDESCENS). Gerhard Fankhauser, Princeton University. (Lantern; 15 min.)

The normal process of sex-differentiation in Triturus viridescens follows the general course described for other species of newts. In undifferentiated gonads, the germ cells have large, deeply lobed, and lightly staining nuclei. In gonads of larvae 12 to 13 weeks old, the appearance of spherical nuclei marks the beginning of ovarian differentiation. This is followed by the formation of a central cavity and of nests of germ cells (oogonia) at the periphery. At the time of metamorphosis, rapidly growing, young oocytes are already present.

Differentiation in male direction progresses more slowly. At metamorphosis, the young testes differ from undifferentiated gonads by the larger number of germ cells, which are scattered throughout the cross section of the gonad, and by slight changes in the appearance of the nuclei. The testes are both shorter and narrower than the ovaries.

Among normal, living larvae of Triturus viridescens, occasional triploid individuals may be found. Four triploids were raised last winter and preserved during or after metamorphosis. In one of these, the gonads appear to be typical testes. The gonads of the other three triploids resemble ovaries, but are much smaller than in normal females; they contain a small cavity, but few oogonia and no growing oocytes.

A satisfactory interpretation of the effects of triploidy on sex will not be possible until triploid gonads in more advanced stages of differentiation have been studied. However, the observations made so far seem to indicate that the effects differ from those described in Drosophila.

82. ANDROGENETIC DEVELOPMENT OF THE FROG EGG, RANA PIPIENS. Keith R. Porter, Harvard University and Princeton University. (Introduced by Leigh Hoadley.) (Lantern; 15 min.)

A technique is described by which the second maturation spindle and so all of the maternal chromatin can be removed from the egg of Rana pipiens following its activation and penetration by the sperm. The operation, as applied to this egg, is considered satisfactory because a large number of eggs can be treated in a short time, the maturation spindle is removed with certainty, and only a slight alteration in the cytoplasmic content of the egg is produced. Between 95% and 100% of the operated eggs undergo first cleavage and of these the majority develop for 8 days, some a few days longer. The haploid nuclear condition persists in the majority of cases over the first 8 days after which it tends to change as indicated by the appearance of scattered diploid and polyploid nuclei. The androgenetic haploids show abnormal and delayed differentiation. Certain of their atypical features as observed externally and internally are described. The cells and nuclei of the haploids are smaller than those of the diploid controls. Yolk disappears more slowly from the cells of the haploids than from those of the diploids.

The ease of production and the success of development of Rana pipiens embryos seems to provide one of the best possibilities so far encountered for the study of haploid morphogenesis and physiology.

83. AN ANALYSIS OF TESTIS AND DUCT SHAPE IN DROSOPHILA. Curt Stern, University of Rochester. (Lantern; 15 min.)

In many species of Drosophila, including melanogaster and azteca, young testes are spherical or ellipsoidal vesicles which later become long spirals or helices. Spiralization depends upon attachment of the testis to a vas efferens originating from an imaginal disc apart from the gonad. Dependence is shown by a) gynandromorphs in which the testes remain ellipsoidal when they are unattached; b) implantation of immature testes into larvae where gonads, regardless of host or donor origin, undergo spiralization only if they become attached to vasa; c) testes in the melanogaster stock in which the ducts are either absent or show various degrees of underdevelopment. Here the testes are spiral only if attached to normal vasa.

In some species such as D. pseudoobscura the testis normally remains ellipsoidal. Xenoplastic transplantations between azteca and pseudoobscura show that the donor testis if attached to a host vas assumes the shape characteristic of the host species. Spiralization is caused by unequal growth of the basal region of the gonad membrane. It is not yet clear whether this growth is induced in the gonadal tissue under the influence of the vas or whether cells of the vas themselves participate in forming the testicular sheath.

The ducts themselves develop as straight tubes but also become helical in later stages. It can be shown that their shape is not dependent on internal growth processes but is a mechanical consequence of the spiral growth of the attached testes.

### 84. Chromosomal deficiencies and embryonic determination in eggs of Drosophila melanogaster. D. F. Poulson, Yale University. (Lantern; 10 min.)

The eggs of Diptera have long been considered among the best examples of determinate (mosaic) eggs in which the pattern of development is established at, or previous to, the time of fertilization. The effects of certain chromosomal deficiencies on the embryonic development of Drosophila melanogaster have a significant bearing on the nature of this determination. Prior to the meiotic divisions and the immediately subsequent fertilization all of the eggs of an XXY female, or of a female heterozygous for an X-chromosome deficiency, are equivalent in potentialities and in cytolplasmic differentiation. Only at the time of fertilization is the genetical constitution of an egg fully determined. If the zygote nucleus receives a normal complement of genes, the subsequent pattern of events is what we call normal development. If, on the other hand, in the case of an XXY female, the attached X enters the polar body and the Y-chromosome remains in the female pronucleus, and the latter is fertilized by a Y sperm, the resulting zygote is deficient for all X-chromosome genes. The development of such a zygote is almost immediately abnormal; although cleavage nuclei undergo synchronous mitoses at the normal rate they fail to become distributed uniformly throughout the egg and remain chiefly in the anterior half. There, at the time for normal migration, they move into the cortical layer. Cell formation is incomplete and no blastoderm is produced. Cytological irregularities are frequent. In cases of smaller deficiencies the effects appear later, but similarly involve disturbances of fundamental developmental processes. Thus in the absence of a full complement of genes the egg is not even an 'embryo in the rough.'

### 85. Cytoplasmic influence in inheritance in Daphnia. A. M. Banta and Thelma R. Wood. Brown University. (Lantern; 15 min.)

The location of genes in chromosomes all but eliminated among geneticists belief that cytoplasm had any place in inheritance. Embryologists, on the other hand, saw in the predetermination in the egg of the type of development, and especially in organizers, major importance in cytoplasmic influence. Geneticists, however, attribute to the chromosomes in the oocytes a determining influence upon the organization of egg cytoplasm and thus a predetermination of the pattern of development. Geneticists also postulate gene determination of organizers. These interpretations would seem to leave nothing to be transmitted primarily by the cytoplasm except inclusions, e.g., plastids in plants.

But geneticists themselves are finding occasional cases which appear to leave some doubt as to the all-sufficiency of chromosomes in inheritance. Our Cladocera studies have provided unmistakable and fairly pronounced differences between offspring from reciprocal crosses, which differences are apparently not due to sex-linked factors and may be interpreted as indirect chromosomal influences through the egg cytoplasm. But we also have, from study of two characters highly variable in their manifestation, groups of data which strongly suggest cytoplasmic effects in transmission—in these cases a relationship between phenotype of mother, independent of genotype, and phenotype of parthenogenetically-produced offspring. Inasmuch as the phenotype of the mother is influenced by environmental factors and similarly reared offspring from phenotypically different, but genotypically identical, mothers differ slightly, these cases suggest the transmission of environmental effects—a conception philosophically unpopular with all of us. Further evidence is being sought.

86. RECENT STUDIES ON AVIAN CHROMOSOMES. Robert T. Hance, University of Pittsburgh. (Lantern; 5 min.)

A number of chromosome counts in a variety of domestic birds has been reported since the publication of my paper in 1926. The counts are generally higher and seem to be due in part to poor preparation and in part to failure to see connecting links between the smallest chromosomes of the series. The latter has led to the counting as separate bodies the chromomeres of single chromosomes. Since the longer chromosomes, as seen by other workers, are in perfect agreement with those recorded by me both in my original and in recent observations, the suggested explanation for the numerical differences seems valid.

87. NEW HISTOLOGICAL METHODS. Robert T. Hance, University of Pittsburgh. (Lantern; 10 min.)

Improved methods will be described that affect the more rapid action of the fixative; better and quicker dehydration with the retention of an unusual softness of the tissue; paraffin mixtures that permit either a rapid penetration or the immersion of tissues for long periods without injury; apparatus for cutting paraffin of low melting points; a better albumin mixture; speeding the staining reactions of several common dyes.

88. SOME PROPERTIES OF LIVING CHROMOSOMES. John B. Buck and Robert D. Boche, Carnegie Institution of Washington, Baltimore. (Lantern; 15 min.)

A method was developed for studying salivary gland chromosomes in the unequivocally living condition. It consists in flattening the living larva between slide and cover glass and observing and measuring the nuclei and chromosomes directly through the body wall under high magnification.

Study of the salivary glands of four genera of Diptera showed that in the normal living condition the chromosomes fill, or nearly fill the nucleus. Nuclear volume thus equals chromosome volume.

In Sciara asphyxiation causes the salivary gland chromosomes to shrink into a spherical clump, the diameter of which serves as an accurate measure of the decrease in volume. Nuclear volume as a whole remains constant, so that the exchange of fluid is entirely intranuclear. The shrinkage of the chromosome is reversible up to a maximum of about 65% loss in volume. Injection of hypotonic and hypertonic Ringer solutions into the larva causes reversible swelling (at least 20%) and shrinking (at least 35%), respectively, of both nuclei and chromosomes. Mechanical pressure on the cytoplasm causes shrinkage of the chromosomes in the affected cells.

In distinguishing between 'living' and 'dead' salivary chromosomes it is instructive to draw an analogy to certain features of protein chemistry. As long as the chromosomes are able to take in and give out fluid they are comparable to native proteins, but the loss of this capacity, which is associated with the death of the organism, resembles the irreversible loss of solubility attendant on protein denaturation.

### 89. OBSERVATIONS ON ABORTIVE OOCYTES IN THE CAMEL CRICKET. M. W. Johnson, Duke University. (Lantern; 10 min.)

Near the beginning of the growth period some of the oocytes show signs of degeneration. Their nuclei become picnotic and the cells tend to round up. These abortive cells at first are wedged in between the definitive oocytes.

Later during the growth period, however, some of them, which have now become quite homogeneous, are found within the cytoplasm of the normal oocytes. The majority of them are ovoid bodies but they may become extremely elongated and irregular. Many of them have been followed into heavily yolked eggs where they still maintain their distinctiveness from the surrounding cytoplasm.

The ovoid bodies may incorporate within their interior small areas of egg cytoplasm. When the definitive ova form yolk, these small areas of isolated cytoplasm also become filled with yolk granules. The fact that these ovoid bodies reveal no Golgi material, and yet yolk forms within them, strongly indicates that yolk in this species is not produced by a direct transformation of Golgi bodies as has been described in various insects.

### 90. THE STRUCTURE OF THE GOLGI APPARATUS IN THE TISSUES OF AMPHIBIA. Arthur W. Pollister, Columbia University. (Lantern; 15 min.)

In all tissues of Amphibia the Golgi apparatus is lamellar in structure. In epithelia it typically approximates the form of a vertical collar, in the distal end of the cell. In leucocytes and fibroblasts it is a horizontal collar, or plate with a central perforation, located near the center of the main mass of cytoplasm. In smooth and cardiac muscle this horizontal collar is greatly elongated. In cells of cartilage and bone, and in odontoblasts the apparatus is a complicated network of narrow strands. There is a complex platework in smaller nerve cells; some larger ganglionic cells contain a large number of separate Golgi platelets. The lamellae are uniform in thickness in all cells, approximating 0.2 $\mu$; and they appear to have the properties of an elastic solid. On the basis of orientation of centrioles and Golgi apparatus all cells of Amphibia may be assigned to one of two groups: A, the epithelial, or physiologically polarized type; B, the leucocyte, or physiologically unpolarized type.

### 91. A CYTOLOGICAL STUDY OF TRANSPLANTED HIND LIMBS OF CHICK EMBRYOS. Herbert L. Eastlick, University of Missouri. (Lantern; 15 min.)

A cytological study has been made of the musculature of normal shaped grafts which were obtained by transplanting hind limb buds of 2- to 3-day chick embryos into the intra-embryonic coelome of hosts of the same age. These transplants are approximately a third smaller than host limbs. They range in age from 8 days of incubation up to a day beyond hatching.

Cytological examination reveals that the muscles do not develop normally. While some striated areas are found in grafts of 10 or more days of age, such regions are quite restricted. Usually most, and not infrequently all, of the muscles are found to have undergone fatty degeneration. A regular order of degeneration seems to occur. Many myoblasts develop striations but the latter persist for a relatively short period of time and soon become indistinct and disappear. This stage seems to be followed by the appearance of fibrous tissue which in turn is succeeded by fat cells. By hatching time fatty degeneration has progressed to such an extent that little or no muscular tissue persists.

The degeneration of the musculature is undoubtedly associated with a lack of normal attachment and the absence of a motor nerve supply.

### 92. THE EFFECT OF INHIBITORS OF CARBOHYDRATE METABOLISM ON THE GROWTH OF THE CHICK FIBROBLASTS. Charles M. Pomerat, Clark University. (Lantern; 15 min.)

By means of a well-established photographic technique for the measurement of the division rate of cell colonies, it has been possible to study the growth promoting activity of embryonic and adult organ extracts upon fibroblasts.

Inhibitors of the respiratory phase of the glucolytic process, such as HCN and mallonic acid, were found not to stop the growth of fibroblasts in the presence of embryo extract. Following the addition of glyceraldehyde 0.002 M, cmplete cessation of cell division was observed. The effect was not obtained with caramelized glyceraldehyde. Hydrolyzed glyceraldehyde and other aldehydes such as acetaldehyde and benzaldehyde at concentrations of 0.002 M also inhibited growth although corresponding acids were not effective. Needham and Lehmann have reported that phosphorylating glycolysis is completely stopped by fluoride at $5 \times 10^{-3}$ M whereas glucolysis is inhibited only about 45%. Furthermore, they found that glucolysis of the embryo is completely inhibited with M/20,000 iodoacetate while M/2000 is necessary to arrest glucolysis of adult muscle. Using suitable concentrations of these inhibitors, it was found that growth of chick fibroblasts in the presence of embryonic extract seemed to be associated with a non-phosphorylating glucolytic process.

### 93. A NEW TYPE OF PROTOPLASMIC MOVEMENT. W. J. Baumgartner, University of Kansas. (Motion picture; 12 min.)

Physiologists and zoologists today recognize four types of movement of protoplasm. They are: 1) slowly flowing within the cell, 2) amoeboid, 3) ciliary and 4) muscular. The first two are slow and the second two are rapid and have specialized structures in the protoplasm. The author has found in the cockroach in what he believes to be blood cells, a very rapid vibrating of one end of the cell. Or it may be an undulating, wave-like-motion of the outer edge of the cytoplasm. The cells do not progress, but they may rotate slowly.

The movement may have some connection with the phagocytic action of these insect blood cells; it certainly explains the numerous shapes described in these protean cells.

The cimenatograph has recorded the motion and will be used to show the movement.

### 94. A QUANTITATIVE STUDY OF THE HISTOLOGY OF THE LYMPH NODES OF THE SUBMAXILLARY REGION OF 20-DAY-OLD ALBINO RATS. J. E. Kindred, University of Virginia. (Lantern; 15 min.)

Data from ninety-eight nodes from nine 20-day-old albino rats form the basis of this study. The nodes range in volume from 20,000 to 1,500,000,000 cu.μ. The smallest nodes (less than 1,000,000 cu.μ in volume) are vascular plexuses invaginating a lymphatic which becomes the peripheral sinus. In the intervascular framework are small lymphocytes, fibroblasts, neutrophils and eosinophils. No mitosis was observed. In the next group (up to 10,000,000 cu.μ in volume) larger lymphocytes are present, few in mitosis, but the vascular character still predominates. A medulla is present in nodes above 10,000,000 cu.μ in volume. In these nodes vasa are numerous, but intervascular distances are greater and there is a concentration of small lymphocytes in the cortex. Cell differentiation is greater in the medulla; and plasma cells and myelocytes are present in small numbers. Also in the medulla, mitosis is greater in all types of lymphocytes than it is in the cortex. Secondary nodules appear in the cortex of nodes with a volume above 100,000,000 cu.μ. The smallest nodules are characterized by small lymphocytes, intermediate small lymphocytes and medium-sized lymphocytes. In the larger nodules, medium-sized, large and giant lymphocytes are more numerous and there is a definite increase in phagocytic reticulum cells. The larger lymphocytes are predominantly in mitosis in the smaller nodules, and as the nodules increase in size in medium-sized lymphocytes increase in mitotic activity.

### 95. THE DUODENUM OF THE TURKEY: HISTOLOGY AND AUTODIGESTION. Lauren E. Rosenberg, University of California. (Introduced by Charles A. Kofoid.) (Lantern; 15 min.)

The duodenum of the turkey has been studied critically to obtain the normal histological picture as a reference standard useful to pathologists.

The cell types are similar to those in mammals, but often vary in size, number and staining reactions. Chief cells of the villi are large. Basal granular cells of the villi, crypts and tunica propria are easily demonstrated by ordinary techniques, but the granules are decidedly amphophilic rather than oxyphilic. The mucous cells are of conventional forms. Paneth cells have not been found.

The eosinophils of the turkey are of two types, one with round granules, the other with crystalloid inclusions. When free in the propria both are amphophilic. Russell-body cells of various sizes are present and appear to be derived from plasma cells.

Typical lymph nodules are confined entirely to the propria of the villi. Both enteric nerve plexuses are within the muscularis. The crypts of Lieberkühn are simple and sac-like. A definite submucosa is lacking; Brünner's glands are absent also.

Between the muscularis mucosae and the broad bundles of circular muscle there is an accessory circular layer not found in mammals.

Autodigestion of the epithelium of duodenal mucosa begins within a few minutes after death; it starts less rapidly in the jejunum and is long delayed in the ileum. For proper fixation of duodenum, it must be removed within 10 minutes after death, opened at once, gently washed with warm saline, and fixed forthwith.

### 96. HISTOLOGY OF THE PAROTOID GLAND OF BUFO A. AMERICANUS HOLBROOK.

Paul L. Carroll and Charles G. Wilber, Marquette University. (Lantern; 7 min.)

The parotoid glands of this toad are located on the dorsolateral surface of the head somewhat posterior to the eyes and tympanic membrane. A preparotoid crest of skin is prominent.

Small portions of the gland were fixed in Bouin's fluid and run up through dioxan. Both vertical and horizontal sections were stained in Harris' hematoxylin and eosin, muci-carmine and Wright's stain.

Two main types of glands are encountered; the small, simple saccular mucous glands, and the large, numerous compound saccular granular glands. The ducts of the mucous glands are short and narrow, while the ducts of the granular glands are long and wide. Some muscle fibers surround these granular glands, but do not seem to be present about the mucous glands. A wavy layer of dense connective tissue separates the two kinds of glands. The epithelial cells in the walls of the mucous glands are larger than those of the granular type. Wright's stain turns the mucous in the glands blue.

Sections of the skin of the frog, Necturus and Amphiuma are briefly considered by way of comparative study.

### 97. CROSS SECTION ANATOMY OF ANODONTA GRANDIS SAY AND OSTREA VIRGINICA GMELIN.

Paul L. Carroll, F. L. Mierzwa and H. J. Barnett, Marquette University. (Lantern; 7 min.)

Small, young clams and oysters were killed, fixed and prepared for sectioning at 10 $\mu$. Ehrlich's hematoxylin and eosin gave the most satisfactory contrast to the various tissues.

After the gross specimen was removed from the shell, it was drawn to scale with accurate indication of all of the superficial structures. A second gross specimen as similar to the first as possible was dissected and a drawing of the exact position of the internal organs was made.

Complete serial sections of both the clam and the oyster were made from the anterior end. The only difficulty encountered was a slight crumbling of the muscles.

Only three sections through each animal are studied at this time; the first is taken at the anterior end and shows the mantle, labial palps and part of the mouth; the second is taken near the middle of the animal and shows especially the position of the digestive and reproductive systems; the third is taken near the posterior end and through the muscle.

These serial sections are especially helpful in bringing out the differences in the internal organization of the clam and oyster.

### 98. MOULTING IN THE CRUSTACEAN, CRANGON ARMILLATUS. Hugh H. Darby, College of Physicians and Surgeons, Columbia University. (Lantern; 15 min.)

The moulting process in Crangon armillatus was found to exhibit the following characteristics. There was a marked diurnal rhythm, with most individuals moulting in the early afternoon. There was no correlation with temperature. The normal length of the intermoult period in uninjured animals was markedly shortened by injury. Animals that had just moulted were unable to harden their shell when placed in oxygen-free sea water. The carapace remained soft for hours afterward. The oxygen demand of newly moulted animals was compared with that of normal individuals between moults, and was found to be twice as high.

The method of breaking the exo-skeleton at the time of moulting was investigated. Preparatory to moulting the carapace is reduced in thickness. Preformed lines of fissure, observable at all times, are then ruptured by hydrostatic pressure which develops within each joint. The puncture of a single joint (without injury to the nerve) so that no pressure could be built up within it resulted in failure to break that particular segment of shell, even though the rest of the carapace was shed without difficulty.

### 99. CHEMICAL CONTROL OF AUTOTOMY IN PETROLISTHES ARMATUS. H. H. Haskin and J. H. Welsh, Harvard University. (Introduced by A. C. Redfield.) (Lantern; 10 min.)

The results of stimulation by grasping in random order the walking legs and chelae of the anomuran, Petrolisthes armatus, indicate that every autotomy influences the responses to succeeding stimuli applied within a short time thereafter. The accumulation of some excitatory substance up to an optimal concentration would account for the rise from 56% autotomies on the first stimulus to 85% on the fourth stimulus. On subsequent stimulation the per cent of autotomies drops rapidly as inhibiting processes set in.

The mediation of the autotomy reflex by a substance, of which some passes into the general circulation without immediate destruction, is indicated by facilitation of further autotomies. The results of injection of eserine, atropine and acetylcholine in appropriate concentrations, all point to the latter as the mediator or excitatory substance. Acetylcholine in high dosage will cause all legs to be dropped without further stimulation. In lower concentration the acetylcholine effect is scarcely discernible from that of eserine in increasing the number of autotomies on the first stimulus. Atropine injection reduces the per cent of autotomies on the first stimulus and presents facilitation of further stimuli but does not prevent the appearance of the inhibitory process. That inhibition of autotomy does not result from increase in the acetylcholine to an inhibitory level is also deduced from the results obtained in the abovementioned injection experiments.

### 100. THE BIREFRINGENCE OF THE SHEATH OF THE EARTHWORM GIANT NERVE FIBERS. G. Wellford Taylor, Washington University, St. Louis. (Introduced by Caswell Grave.) (Lantern; 15 min.)

It has long been known that the inner sheath surrounding the axon of the giant fibers contains oriented lipide molecules. This is indicated by the fact that the sheath shows birefringence which is negative with respect to the long axis of the fiber.

Schmitt and Bear have shown that in vertebrate 'myelinated' fibers the birefringence of the sheath is a function of the diameter of the fiber end, to a certain extent, of the velocity of conduction in the fiber. The present work furnishes similar quantitative data for the birefringence of the sheath of earthworm giant fibers and for the variation of this birefringence with fiber diameter.

Birefringence was measured in teased fibers with λ/20 Köhler rotating plate compensator, values being calculated according to the relation derived by Schmitt and Bear.

The birefringence increases with increase of fiber diameter; the value for 26 $\mu$ fibers being about 0.0005 and for 60 $\mu$ fibers about 0.001. Frog sciatic fibers which conduct with velocities about equal that of earthworm giant fibers (about 20 meters per second) would possess sheaths with a birefringence more than ten times that of the giant fibers. High velocity of conduction is presumably attained in the latter by virtue of the large diameter of the axon.

In a few cases the segmental partitions of the giant fibers appeared to show very weak birefringence which was negative with respect to the long axis of the partition, suggesting the presence of lipide molecules oriented normal to the surface of the partitions.

### 101. PRELIMINARY EXPERIMENTS ON THE COLOR CHANGES OF THE AMERICAN CHAMELEON, ANOLIS CAROLINENSIS (CUVIER). F. H. Wilson, Tulane University. (Lantern; 10 min.)

These animals with green cellophane filters fitted over their eyes turn green and stay green when exposed to daylight or artificial light. If black hoods are fitted over the eyes, so as to exclude light from the eyes, the animals become a deep chestnut brown when exposed to daylight, artificial light or to light which has passed through a green cellophane filter. With eyes uncovered and the animal exposed to daylight or artificial light it changes to a yellowish brown, and when placed in darkness under ordinary summer temperatures it becomes green. The color changes are thus influenced by the quality of light entering the eyes. Light either daylight or artificial or having passed through a green cellophane and acting only on the skin can bring about the brown phase. Green light entering the eyes inhibits to varying degrees the effects of light on the skin.

The parietal eye does not function as a light receptor influencing color changes.

### 102. THE PHYSIOLOGY OF COLOR CHANGE IN THE WINTER FLOUNDER, PSEUDOPLEURONECTES AMERICANUS. Clinton M. Osborn, Harvard University. (Introduced by G. H. Parker.) (Lantern; 12 min.)

The winter flounder exhibits the adaptive responses to illuminated black and white backgrounds typical for various fishes. In total darkness, a fish ultimately assumes an intermediate shade even when previously adapted to black or white. Freshly denervated areas, which on illuminated backgrounds darken immediately, and then gradually fade to the general body color, remain black in total darkness. Excitement causes marked blanching of denervated areas, temporary pallor of the innervated melanophores of black-adapted fishes, but darkening of the innervated cells of white-adapted fishes. White spots on black-adapted fishes and black spots on white-adapted fishes disappear on excitement. Observable effects of adrenalin injection into black-adapted fishes are similar to excitement responses. A denervating cut in a fish previously injected with adrenalin or vigorously excited will not produce a dark band until the effect of the earlier stimulus has worn off. It is believed that an adrenalin-like substance secreted by the flounder produces the pallor in denervated areas following excitement. Doses of pituitary extract containing sufficient melanophore-dispersing principle to darken three frogs for 12 hours failed to produce significant darkening in pale fishes and were incapable of overpowering the excitement pallor of denervated areas in black-adapted fishes.

These findings, together with observed effects of certain drugs, make it appear that the rapid responses of localized areas and of the whole integument are brought about by nervous stimulation and the more gradual responses (especially paling) may be reinforced by an adrenalin-like substance.

### 103. THE ILLUMINATION OF THE EYE NECESSARY FOR DIFFERENT MELANOPHORIC RESPONSES OF FUNDULUS HETEROCLITUS. Earl O. Butcher, Hamilton College. (Lantern; 10 min.)

When fish are in a black dish, darkening of the fish's body is induced by illuminating the lower region of the eye with either diffuse light or light from a mazda lamp. However, only diffuse illumination of the upper region of the eye induces paling of the body. By placing fish over illuminated circles of white paper of various diameters, it has also been found that the illuminated area of the upper region must reach a certain size before the body pales. Fish placed over semicircles of white paper fail to pale because illumination of both lower regions of the eyes in this instance is enough to inhibit the paling response induced by the reflected light to only one upper region.

The brightness of a gray background receiving 50 foot candles from above was 3 foot lamberts. Such light relations induced fish to assume a gray shade. When the gray background was illuminated from below, so that its brightness became 10 foot lamberts, the fish paled quickly.

Fish, therefore, assume the shade of a gray background because of the ratio between the reflected light from this background and the direct light from above. A ratio is involved because illumination of one region of the eye tends to diminish the response induced by the simultaneous illumination of the opposite region.

### 104. Reactions to light and the photoreceptors in certain decapod crustaceans. Walter N. Hess, Hamilton College. (Lantern; 10 min.)

A study was made of the regional photosensitivity of the body of the common crayfish, the American lobster, the Florida lobster and Crangon armillatus. Using a given light intensity it was found that photosensitivity in all of these animals varies with a) age of the animal, b) region of the body stimulated, c) amount of pigmentation, d) degree of dark adaptation and e) the general physiological conditions of the animal.

In general, the degree of photosensitivity in all of these animals varies inversely with the amount of pigment in the external skeleton. Mature animals that have hard pigmented external skeletons are sensitive to light only in those regions containing very little or no pigment. Freshly molted animals are sensitive to light in many regions of their bodies including the antennae, telson, uropods as well as the dorsal and lateral regions of the cephalothorax and abdomen. The degree of photosensitivity is greatest in young animals, and in those that have recently molted. A study has been made of the neurosensory structures in these photosensitive regions.

### 105. Some aspects of the relative roles of increased periods and constant periods of illumination in the sexual photoperiodic activation of the male starling. J. Wendell Burger, Trinity College. (Lantern; 15 min.)

Male starlings, sexually inactive on articial day lengths of 6 hours failed to show spermatogenetic activity after 40 days when their daily light ration during this time was gradually increased to 9 hours. These same starlings began spermatogenesis when their daily light ration was increased from 9 hours to $9\frac{3}{4}$ hours over a 16-day period. Testes of both starlings and juncos which were beginning to form primary spermatocytes, failed to continue their spermatogenetic development when the birds were given constant lengths of day of $10\frac{1}{4}$ hours. It is concluded that: a) periodic increases in length of day are effective in inducing spermatogenesis in the male starling only when the individual day lengths are greater than 9 hours; b) a constant daily ration of light ($10\frac{1}{4}$ hours) will not permit spermatogenetic development, even though this daily ration is above the minimum 9-hour threshold; c) in the photoperiodic sexual activation of the male starling both periodic increases in day lengths and individual days longer than 9 hours must be present before spermatogenesis will occur.

### 106. Effect of light and darkness on recovery in Peranema trichophorum after response to light. Landrum B. Shettles, Johns Hopkins University. (Lantern; 15 min.)

In light of 6458 m.c., the exposure period of dark adapted peranemas is 2.6 seconds, and the energy required for response is 16791 m.c.s.; after 5 seconds in the light plus 15 seconds in darkness following the response, the former is 1.4 seconds, and the latter, 9041 m.c.s. As the time in the light following the response increases, the exposure period and energy required for response decrease to minima of approximately 0.8 second and 5000 m.c.s. at about 45 seconds exposure, then remain fairly constant. This shows that recovery does not occur in the same luminous intensity as that used to induce the response, and that the extent of recovery varies inversely with sensitivity to light, but directly with the energy required for response.

If dark adapted peranemas which have responded to 4305 m.c. are subjected to darkness, they recover completely in about 45 seconds.

If dark adapted peranemas which have responded in 4305 m.c. are subjected to 3229, 2152, 1076, 538 or 323 m.c., they do not recover, but if they are subjected to 269 m.c., recovery increases to about 50% at 45 seconds exposure, then remains nearly constant. Thus, recovery after response to light of a given intensity occurs partially in certain lower luminous intensities.

These results indicate that increase in light induces changes in the animal which are partially or wholly eliminated in certain lower intensities or darkness.

### 107. MECHANISMS INVOLVED IN GROUP PROTECTION OF EUPLANARIA DOROTOCEPHALA FROM ULTRAVIOLET RADIATION. Janet Wilder, The University of Chicago. (Introduced by Frank R. Lillie.) (Lantern; 15 min.)

As we reported last year Euplanaria can survive the effects of irradiation with ultraviolet longer, other things being equal, if more rather than fewer are exposed together. After exposure under conditions of equal numbers, worms cytolyzing in groups survive longer than those cytolyzing singly. All assays were conducted in fresh, non-irradiated water into which the grouped and isolated worms were transferred alternately.

The group effect was apparent when worms were placed in volumes proportional to numbers present providing surfaces were equal and depth unequal but not if surfaces were unequal and depths equal.

An artificial increase in carbon dioxide, to simulate a probable condition in the water about grouped worms, delays cytolysis following irradiation. Agitation of the worms hastens cytolysis and causes the usual differential survival of grouped and isolated worms to disappear. Irradiated planarians cytolyze more slowly in slightly acid than in slightly alkaline water.

Irradiation increases the permeability of planarians to contained electrolytes if the worms are assayed in hypotonic water. This effect was not found in water with a conductivity of $325 \times 10^{-6}$ mhos or more. As little as 0.000001 M calcium chloride in distilled water delayed cytolysis; grouped planarians probably give off enough more calcium than do isolated individuals to produce this effect. It is probably not a factor in water already fairly rich in calcium.

We are not prepared to delimit precisely the roles played by calcium, H-ion concentration, and carbon dioxide in producing longer survival of grouped planarians.

### 108. THE INFLUENCE OF THE PUPILLARY LIGHT REFLEX UPON DARK ADAPTATION MEASUREMENTS. Charles Haig, Research Division for Chronic Disease, Welfare Island, and Department of Medicine, College of Physicians and Surgeons, Columbia University. (Lantern; 15 min.)

Measurements of the dark adaptation of two subjects, made after varying degrees of light adaptation, and using a constant pupil, show regularities not exhibited by measurements made with the natural pupil. The effect of constant pupil area is to prevent distortion of the function (log threshold intensity versus time in the dark) introduced by variations in the size of the natural pupil.

The influence of the pupillary reflex is especially marked on cone measurements and on those of the rods following low degrees of light adaptation. Measurements made with the fixed pupil are found to have the same mathematical form independently of the degree of light adaptation, whereas those made with the natural pupil vary in form when the degree of pre-adaptation is altered.

It is recommended that a fixed pupil be employed for dark adaptation measurements wherever the speed or form of the function is important.

### 109. THE YELLOW PIGMENT OF THE MEXICAN BEAN BEETLE, EPILACHNA VARIVESTRIS. MULS. Nellie M. Payne, American Cyanamide Company. (Lantern; 15 min.)

The yellow pigment of both the body and the eyes belongs to the carotinoide group. Pigment is deposited in the elytra during their formation. The degree of pigmentation depends upon the age of the beetle and upon the temperature. The pigment appears to be derived from a plant source since it is identical to that found in the bean leaves upon which the beetle feeds.

### 110. THE TOXICITY TO HONEYBEES OF ACID LEAD ARSENATE, CALCIUM ARSENATE, AND PHENOTHIAZINE IN RELATION TO PARTICLE SIZE. Lloyd M. Bertholf, Western Maryland College and Bureau of Entomology and Plant Quarantine, United States Department of Agriculture. (Lantern; 10 min.)

Previous work has shown that when the ordinary commercial preparations of these three insecticides are fed to bees, calcium arsenate is most toxic, lead arsenate less, and phenothiazine very much less. In the present work two or three different sizes of particles of each of these three insecticides were tested. The materials were suspended in 65% sugar syrup, and measured amounts of the suspensions were fed in single doses individually to honeybees of known age, after which the bees were placed in cages provided with feeders for sugar syrup and water, and the cages placed in an incubator at constant hive temperature and low humidity until all the bees died. The number dead was recorded daily and the average longevity of each lot computed.

The results showed that the toxicity to bees of these materials, especially lead arsenate and phenothiazine, is decidedly greater in the form of small-sized particles, the large-sized particles of the latter two being almost entirely harmless. The toxicity of calcium arsenate is greater than either of the others and depends less upon particle size. This is assumed to indicate its greater solubility in the slightly acid digestive juices of the bee.

Measurements of the average longevity of lots of control bees, ranging from ½ day to 80 days of age at the time of their confinement in cages, showed a maximum when this age is about 16 days. The presence or absence of a queen in the cage, and the allowing of cleansing flights made little difference.

111. HOMOLOGY AND ANOLOGY. Alan Boyden, Rutgers University. (Lantern; 12 min.)

The biological significance of homology is now relatively clear. This is not true of analogy. Analogy has been used to mean 1) a superficial similarity in structure, 2) a fundamental similarity in structure, 3) a similarity in function of organs whether homologous or not. Consequently analogy has now no definite meaning and little value. It could be 1) dropped completely, 2) employed to mean a superficial similarity in structure, 3) employed to mean a similarity in function. The latter usage seems preferable for these reasons, 1) without introducing new terms the chief kinds of similarities of importance to the zoologist may be clearly described, 2) there is no false implication that analogy and homology are antithetic nor that 3) superficial structural similarities are 'due to' use. Homologous and analogous would then have meanings as follows: 1) homologous, fundamentally similar in structure, 2) non-homologous, not so, 3) analogous, similar in function, 4) non-analogous, not so. These terms could be used in combinations, as follows: 1) homologous and analogous—arm of man and ape, 2) homologous and non-analogous—arm of man and fore limb of elephant, 3) non-homologous and analogous—arm of man and cheliped of crab, 4) non-homologous and non-analogous—arm of man and tail of tadpole. Combination (4) would be seldom used for such organs have least in common. But all the combinations are absolutely clear in their meaning and the first three are highly significant to every zoologist.

112. EVOLUTIONARY ALLOMETRY IN THE SKELETON OF THE DOMESTICATED DOG. Hyman Lumer, Western Reserve University and Fenn College. (Lantern; 15 min.)

The relative growth of function $y = bx^{\alpha}$ was applied to measurements on the skull and limb bones in adult specimens of twenty-eight modern breeds of dogs. The data conform to the equation, yielding for some relationships a single curve including all the breeds, and for others two or more distinct curves. The greatest diversity occurs in the various width-length relationships in the skull, for each of which five distinct curves were obtained. The groupings of the breeds with respect to these curves are the same for all the relationships concerned. Prehistoric and ancient types, according to the available data, fall on the same curves as the modern breeds. These results indicate that the species Canis familiaris is divisible into five subgroups, or allometric tribes. The group curves may be termed curves of evolutionary allometry.

The occurrence of such group curves is most simply explained on the assumption that ontogenetic relative growth is essentially the same in all breeds within a given allometric tribe. This hypothesis is supported by the fact that $b$ and $\alpha$ for certain growth relations in the German sheep dog are the same as those for the corresponding group curves. If the assumption is correct, the evolution of any tribe must have involved mutations affecting adult body size but not the relative growth of the parts in question.

### 113. THE DUCTUS GENITO-INTESTINALIS IN THE GENUS CASTRADA BASED UPON CASTRADA LUTHERI N.SP. William A. Kepner, M. A. Stirewalt and F. F. Ferguson, University of Virginia. (Lantern; 5 min.)

None of the species of Castrada is known to have a ductus genito-intestinalis, though Luther in 1904 and Hofsten in 1911 described a small sac, the former in C. viridis and the later in C. fuhrmanni, for which they could find no homolog.

The present authors have found a ductus genito-intestinalis in C. lutheri, which presents homologous features with the small sac of both C. viridis and C. fuhrmanni. These sacs, therefore, in the last two species are vestigial ducti genito-intestinales.

### 114. A METHOD OF EXCRETION IN MICROSTOMUM, O. SCHMIDT 1848. M. A. Stirewalt, University of Virginia. (Introduced by Wm. A. Kepner.) (Lantern; 5 min.)

The existence of a protonephridial system in the genus Microstomum having been described only in Europe and there only by six of the numerous investigators of the species, a study was made to ascertain the method of excretion in the members of the genus in the United States. No protonephridia have been found. Certain amoeboid cells, however, may be found to move through the parenchyma into the epidermis where they orient themselves. These cells contain waste material of both liquid and solid nature. This material is passed to the outside of the body by loss of the amoebocytic cells which have handled it.

Such a method of excretion is directly comparable to microstomum's handling of the nematocysts of Hydra.

### 115. GRAFTING EXPERIMENTS DIRECTED TOWARD ASCERTAINING THE LOCATION OF FACTORS OF HEAD REGENERATION IN THE EARTHWORM. Ben T. Painter, Harvard University. (Introduced by Herbert W. Rand.) (Lantern; 15 min.)

Factors which determine the occurrence and nature of regeneration in the earthworm may be situated in one or more of the following parts of the animal: digestive tube, nerve cord, and body wall. The experiments consisted in 1) transplanting one or more of these parts from an anterior head-forming region to a more posterior region where heads do not, but tails may, regenerate, and 2) transplanting parts from the more posterior into the head-forming region. The object was to effect all possible interchanges of parts between the two regions. Transplantation of body wall consisted in slipping a sleeve of posterior body wall including the posterior nerve cord over the pharynx, or a sleeve of anterior body wall over the intestine behind the clitellum. In the latter case the graft was made either with no central nervous parts present, with the anterior nerve cord present, or with the posterior nerve cord present. These experiments lead to the conclusion that the intestine and posterior nerve cord can participate in head formation as readily as the pharynx and anterior nerve cord, and that the epidermis of the anterior body wall contains the chief head determiner.

### 116. REGENERATION AND REGULATION OF MULTIPLE HEADS IN PLANARIA MACULATA AND PLANARIA AGILIS. Frank M. Hull, University of Mississippi. (Lantern; 10 min.)

This paper records the results from experiments on several hundred planarians. The longitudinal splitting of the planarian body, posterior to the head resulted in the formation of a head in the crotch of the cut in almost 100% of the cases, provided the raw surfaces were not allowed to heal together. The regulation of this crotch-headed double planarian was of several kinds: 1) exhaustion or death of one or both thus grown together; 2) separation of the two bodies at or near the head region; 3) the complete dominance of activity of one head over the other head and over the two bodies; 4) absorption of one body. The same incision, modified to the extent that the tail was not severed, likewise produced heads in the crotch and sometimes in the posterior crotch. Two longitudinal cuts, dividing the body of the planarian into three longitudinal strips, but without dividing the head or tail resulted in a spontaneous burst of heads or cephalism, greater than any previously reported for this type of alteration. As many as eight heads were found on one planarian body. Four stages of regulation were observed on this eight-headed animal.

### 117. CANNIBALISM IN PLANARIA. Frank M. Hull, University of Mississippi. (Lantern; 5 min.)

Cannibalism became common in mixed cultures of Planaria maculata and Planaria agilis which had been kept for 6 months or longer in large porcelain bowls. The aggregations of individuals were as high as 600 per liter of water. The affected individuals characteristically curved the entire body in a close fitting circle about the victim. Often a compact ball-like mass of planarians first showed where an individual was being destroyed. Wounded and partially eaten planarians usually recovered. These cannibalistic individuals appeared to display an insatiable appetite for their victim.

### 118. THE FOOD CHAIN IN A SMALL TEXAN CAVE. Lorus J. Milne, Randolph-Macon Woman's College. (Lantern; 5 min.)

The macroscopic animal life of a number of caves between Georgetown and New Braunfels, Texas, was investigated from the standpoint of food interrelationships. It was found that the true cave forms were dependent for their nourishment on the bats which slept and defecated in the cave during the day. One ectoparasitic fly was found, apparently accidentally separated from its host. The other forms did not live at the bats' expense. A fungus growing on the nitrogen-rich bat droppings served as food for hordes of cave crickets. Two species of predaceous bugs and a centipede ate the adults and young of the crickets. Dead bats and insects falling into the wetter parts of the caves were attacked by a white flatworm and by microscopic organisms in the water. There was some evidence that the crickets varied their fungus diet with dead insects and bats falling to the drier parts of the cave floor.

119. OBSERVATION ON SOME LOWER TURBELLARIA OF THE EASTERN UNITED STATES. E. Ruffin Jones, Jr., College of William and Mary. (Introduced by the Secretary.) (Lantern; 10 min.)

A study of the Acoela, Rhabdocoela and Alloecoela from various parts of Virginia, North Carolina and Massachusetts has been undertaken. While the work is still in its early stages, nine species hitherto unreported from this country have been identified, including under the order Rhabdocoela: Macrostomum stylopencillum, a new species from North Carolina, Omalostomum schultzei from Massachusetts and Virginia; Dalyellia gilesi a new species from Virginia; Byrsophlebs graffi from Massachusetts; Maehrenthalia agilis from Massachusetts; Solenopharynx flavidus from Massachusetts and Virginia; Sopharynx oculatus from Massachusetts; Polycystis mamertina from Massachusetts; and under the order Allocoela: Enterostomula graffi from North Carolina. In the last-named form the presence of the ductus spermaticus and vagina externa necessitates its removal from the genus Monoophorum and its inclusion in the genus Enterostomula. The excretory system of this species has been observed for the first time, and the excretory tubules have a type of construction which is believed to be unique. A study has also been made of a form which was incompletely described by Graff in 1911 as a new species, Monoophorum triste, from Massachusetts. This study disclosed that this species should be transferred to the genus Cylindrostoma since it lacks vagina, ductus spermaticus and bursa seminalis.

## DEMONSTRATION PAPERS

120. CINEPHOTOMICROGRAPHS OF SKELETAL AND CARDIAC MUSCLE FIBERS IN ACTION. Carl Caskey Speidel, University of Virginia. (Motion picture; 15 min.)

These motion pictures portray microscopic changes in striated muscle fibers throughout contraction, relaxation and other varieties of tension disturbances. (See also abstract no. 75.)

121. A NEW TYPE OF PROTOPLASMIC MOVEMENT. W. J. Baumgartner, University of Kansas.

See abstract no. 93.

122. EFFECTS OF ULTRACENTRIFUGING AND COLCHICINE ON VARIOUS TYPES OF CELLS. H. W. Beams and R. L. King, State University of Iowa.

A series of demonstrations of various animal and plant cells to show the effect of ultracentrifuging and colchicine treatment, and subsequent recovery therefrom.

123. VISIBLE ORGANIZATION OF THE SALIVARY GLAND CHROMOSOMES OF DIPTERA. C. W. Metz, Carnegie Institution of Washington.

Further evidence is presented supporting the view that the visible organization of these chromosomes is that of an alveolar system, superimposed, of course, on the pattern of transverse disks. In different genera, and under different conditions, the alveolar system gives different optical patterns of lines, including honeycomb patterns easily distorted into crisscross or latticework. Comparative studies on four genera indicate that this is responsible for the fact that observers working on three different genera have given three distinctly different interpretations based on the assumption that the visible lines here are chromonemata.

Aside from visible evidence of alveolar or honeycomb organization, the nature of the lines themselves indicates that, even though often appearing thread-like in distorted chromosomes, they cannot represent chromonemata, because they 1) typically form a diagonal crisscross pattern at all optical levels, in side view, 2) regularly intersect at the granules, 3) are often continuous diagonally across both chromosomes of the pair, 4) are far less numerous than chromonemata should be, and 5) differ widely in number in different parts of a chromosome.

Likewise, the evidence indicates that the so-called 'chromomeres' here are not biological units. Some are heavy-walled droplets, each apparently made of material from two independent disks, one of which may be removed by a 'deficiency.' Others are granules of chromatin which may be similarly compound and which sometimes lie between the 'chromonemata.'

Evidence for visible chromonemata is mainly based on distorted chromosomes. Artificial distortion of cytoplasm duplicates pictures seen in distorted chromosomes.

### 124. ALTERATION OF MITOCHONDRIAL PATTERN IN THE HEPATIC CELLS OF THE CHICK EMBRYO. Albert J. Dalton, Western Reserve University and McGill University.

Previous experiments have shown that the injection of insulin into the allantoic cavity of 7- or 8-day chick embryos causes a rapid fall in the blood sugar level and a concomitant excessive storage of glycogen in the hepatic cells. The suprarenal cortical extract, Eschatin, on the other hand, induces glycolysis accompanied by a definite rise in the blood sugar level.

Whether glycogenesis or glycolysis is induced, the mitochondria of the hepatic cells react in a similar fashion. Twenty hours after the injection of either insulin or Eschatin many of the normally filamentous mitochondria of the hepatic cells have been transformed into spherical and short rod-like forms. Five days after the injection of Eschatin, spherical forms are still numerous, while 10 days after the injection, the mitochondria have reverted to their normal form.

It is suggested that these deviations from the normal mitochondrial pattern are associated with changes in cellular activity as such and are not related to any specific action of mitochondria in carbohydrate metabolism.

### 125. DEMONSTRATION OF THE CHROMOSOMES OF THE CHICK. Robert T. Hance, University of Pittsburgh.

Photographs and slides will be used to illustrate the reason for the belief of some workers that the chromosome number in the chick is more than thirty-five or thirty-six as reported in 1926 by the writer.

### 126. DEMONSTRATIONS OF NEW HISTOLOGICAL METHODS. Robert T. Hance, University of Pittsburgh.

A variety of tissues prepared by new techniques will be shown. These techniques have worked well in the preparation of hard fibroid tumors, the muscles of earthworms, general histological and cytological materials of plants as well as of animals. As far as feasible the methods will be demonstrated and directions for carrying out the techniques will be available.

### 127. Morphogenesis of transplanted hind limbs of chick embryos. Herbert L. Eastlick, University of Missouri.

Hind limb bud primordia of 2- to 3-day chick embryos were transplanted into the intra-embryonic coelom of chick hosts of the same age and well formed, normal appearing limbs were secured in approximately half of the successful operations. The hosts range in age from 4 days after the operation up to 1 day beyond hatching. In all cases the grafts are attached to the mesenteries of the intestines, frequently by but a single strand.

All skeletal elements are present and, upon macroscopic observation, the musculature appears fairly normal. However, upon microscopic observation it was found that the muscles of the older grafts had undergone extreme fatty degeneration. This fact coupled with the probable lack of a nerve supply explains the lack of functional activity in the grafts.

### 128. Some effects of mammalian hormonal substances on the differentiation of the gonads of an undifferentiated race of Rana catesbiana tadpoles. William O. Puckett, Princeton University.

A race of Rana catesbiana tadpoles possessing undifferentiated gonads during a part of larval life will be described. These animals possess a type of gonad called a progonad. Late in larval life such progonads differentiate either into the definitive ovaries or testes of the adult frog. A precocious metamorphosis and differentiation of the gonads can be produced by the injection of mammalian pituitary extracts over a period of 25 to 30 days. Such treatment results in the differentiation of approximately equal numbers of ovaries and testes. Injection of the synthetic male sex hormone, testosterone, propionate, in addition to a pituitary preparation brings about the differentiation of typical testes in 100% of the animals of an experimental group. Similarly, injection of the female sex hormone, theelin, in addition to a pituitary preparation results in the differentiation of ovaries in 100% of the experimental animals. Injection of the male or female sex hormone alone does not bring about a precocious differentiation of the gonads.

Microscopic preparations and photomicrographs illustrating the experiments described above will be exhibited.

### 129. Delayed fertilization and development of the guinea pig ovum through the sixteenth day of pregnancy. Richard J. Blandau and William C. Young, Brown University.

By means of artificial insemination prior to and shortly after ovulation in 586 female guinea pigs, it was found that a progressive delay of fertilization caused an increase in sterile inseminations, a decrease in litter size, a decrease in normal pregnancies and an increase in abnormal pregnancies ending in abortion. No normal development followed inseminations 20 hours after ovulation. All the late-fertilized ova which were removed shortly before implantation showed some abnormality, such as general cytolysis, fusion of the blastomeres or retardation in the rate of development.

The ability of many late-fertilized ova to become implanted was evident 1) from the number of abortuses obtained from the experimental series, 2) from the fact that some normal young were born to females inseminated as long as 20 hours after ovulation, and 3) from the study of late-fertilized ova removed from the eighth to the sixteenth day of pregnancy or shortly after implantation. A number of these late-fertilized, implanted ova, studied microscopically, showed gross structural abnormalities and were undergoing reabsorption or were about to be aborted. Other implanted ova showed a marked retardation in growth, but the general cellular structures appeared normal. That these retarded embryos are capable of continued development is suggested by the normal appearance of the uterus and corpora lutea.

The average number of late-fertilized ova found prior to implantation was greater than the number found shortly after implantation, indicating the elimination of ova during the implantation process.

### 130. COMPARATIVE VERTEBRATE HEMATOLOGY. H. E. Jordan, University of Virginia.

Plates of water color drawings of the blood cells of hagfish, lamprey, lungfish, bowfin, skate, dogfish, goldfish, salamander, frog, horned toad, pigeon, opossum and rat.

### 131. MODEL OF A 2-MM. HUMAN EMBRYO. J. L. Platt, University of Virginia. (Introduced by J. E. Kindred.)

The demonstration consists of sections and photographs of a well-preserved human embryo 2 mm. long together with reconstructions of the body and heart. The embryo exhibits two abnormalities not noted in the available descriptions of other young human embryos: 1) the entire heart is placed cephalically; 2) the celomic cavities communicate with each other in the dorsal mid-sagittal region of the body. The structures which may be easily recognized are: two-chambered heart, sinus venosus, dorsal aortae limited to the head, umbilical and vitelline veins distended with nucleated red blood cells, neural groove and folds, notochord, pharynx, first branchial pouches, anterior and posterior intestinal portals, allantois, pleuroperitoneal canals, primitive node and groove, and embryophore. The dimensions are as follows: neural groove, greatest width 0.3 mm., length 0.7 mm.; pharynx, greatest length 0.3 mm., greatest width (at first branchial pouches) 0.15 mm.; heart, greatest length 0.44 mm., greatest width 0.42 mm.

### 132. ANALYSIS OF PITUITARY SUPPORT OF GROWTH OF BODY AND VISCERA IN PIGEONS. J. P. Schooley, O. Riddle and R. W. Bates, Carnegie Institution.

Charted data from 272 hypophysectomized and forty-six normal pigeons (White Carneaux, aged 2.3 months) permit recognition of the particular anterior pituitary hormone, and combination of hormones, which sustain or increase weight in body and viscera (intestine, pancreas, liver). Weights of crop sacs, thyroids, adrenals and testes, obtained concurrently on these same animals, indicate the presence or absence or prolactin, thyrotropin, 'adrenotropin,' and gonadotropin in the various pituitary preparations used in this study.

Prolactin has special and marked ability to sustain and increase weight of body and viscera in pituitaryless pigeons; and appetite, as measured by food consumed, is increased more by prolactin than by any other single pituitary hormone that is available for study. However, prolactin admixed with other pituitary hormones is more effective in inducing growth and splanchnomegaly than is an equal quantity of prolactin alone. Mixtures of gonadotropin and thyrotropin, free from prolactin, do not sustain body weight nor weight of visceral organs in either normal or pituitaryless pigeons. Intermedin has no power to sustain weight in any organ studied. A trypsin digest of a whole (unfractionated) anterior pituitary extract has no ability to sustain weight in any of the above-mentioned organs.

### 133. THE INTERRELATIONSHIP BETWEEN RETARDED GROWTH, AGING AND ULTIMATE SIZE OF THE BODY.

C. M. McCay, L. A. Maynard, Gladys Sperling and Leroy Barnes, Cornell University.

A new experiment in which rats were retarded for periods as long as 1000 days confirms earlier findings that the life span can be extended by decreasing the rate of growth. This retardation of growth was affected by feeding diets that were adequate qualitatively but deficient in calories. Various groups of rats were restricted for periods of 300, 500, 700 and 1000 days. The latter period is well beyond the normal life span of the white rat. After such periods of retardation the animal still retains the power to grow but in some cases this power is beginning to fail after retardation for 1000 days. The male always tends to attain a larger body size than the female even though they have been maintained at the same body size for a period beyond the normal life span. Photographs and charts will be presented to show that the ultimate body size of an animal is decreased in direct proportion to the period of retardation.

### 134. REVIVAL OF ORGANISMS VITRIFIED AT 190°C. BELOW ZERO.

Basile Luyet, Eugene Hodapp and Gregory Thoennes, St. Louis University.

An aqueous colloid freezes when it is exposed to temperatures from 0° to about —15°C.; at lower temperatures it takes the vitreous state, that is, it becomes hard and breakable but its water does not crystallize. Previous experiments with plant material have shown that vitrified protoplasm retains its vitality, while frozen protoplasm is killed. Using the same methods of investigation with animal cells and tissues, we were able to revive frog's spermatozoa and muscle fibers after immersion in liquid air at —190°C. The vitality of the spermatozoa is judged by their motility and that of the muscular fibers by their response to electric stimulation. The essential condition for revival is to traverse the dangerous zone of crystallization temperatures (from 0° to —15°) rapidly enough to avoid congelation. This should be done during rewarming as well as during cooling. The procedure usually requires: 1) A previous reduction of the water content of the material; 2) The use of very minute quanitities exposed in very thin layers to the temperature changes; 3) A sudden immersion in liquid air followed, in due time, by a sudden immersion in a liquid warming bath. (The demonstration will include the vitrification of some colloidal solution, the revival of frog's spermatozoa and the revival of frog's muscle fibers.)

135. TRACING THE PATH OF SPERMATOZOA THROUGH THE SUBSTANCE OF THE FROG'S KIDNEY BY MICRO-INJECTION OF VASA EFFERENTIA. Roberts Rugh, Hunter College.

See abstract no. 11.

136. MICROSTOMUM FEEDING UPON CHLOROHYDRA. Wm. A. Kepner, M. A. Stirewalt and F. F. Ferguson, University of Virginia

Microstoma without nematocysts will be fed hydras and then set aside. The next day, it will be demonstrated that these microstomas have nematocysts oriented at their surfaces. They will again be fed hydra tissues. Three to 6 hours after the second feeding the specimens will be fixed and handed to observers who may take them for sectioning and staining. These sectioned specimens should show nematocysts in various tissues of the rhabdocoele as well as at the epidermis.

137. A MONOGRAPH OF THE GENUS MACROSTOMUM O. SCHMIDT 1848. Frederick Ferdinand Ferguson, University of Virginia. (Introduced by W. A. Kepner.)

This volume is a comprehensive survey of all the information upon Macrostomum up to the present. Stress has been placed upon the following subjects: history, classification, ecology, methods, anatomy, species descriptions, sexuality, egg and sperm-cell development, embryology, physiology and experimentation. The genus at present contains forty-four species, twenty-six of which have been added by the author. An extensive list of references accompanies the text. The original drawings and a culture of Macrostomum will be displayed.

138. RACIAL DIFFERENCES WITHIN THE SPECIES CHAOS DIFFLUENS MÜLLER, THE COMMON LABORATORY AMEBA. A. A. Schaeffer, Temple University.

See abstract no. 46.

139. A MOSAIC COLOR PATTERN IN THE MEXICAN BEAN BEETLE. Nellie M. Payne, American Cyanamide Company.

The usual color pattern of the Mexican bean beetle, Epilachna varivestris Muls., is yellow background for both body and elytra. Superimposed on the elytra are eight black spots arranged in rows of three, three, and two, respectively, from anterior to posterior. The prothoracic shield is generally immaculate. In the specimen exhibited the left elytron has the usual markings. The right elytron has a band which may represent the three coalesced most anterior spots, a longitudinal band along the mesal margin of the elytron, and two discrete spots. The longitudinal band is united to an irregular black area which may represent the median spot of the second row of dots. The right half of the prothoracic shield has a black border; the left half is immaculate.

### 140. THE SEALING OF DEMONSTRATION SPECIMENS IN PLASTIC CONTAINERS. Elbert C. Cole, Williams College.

Containers were made of the plastic, methyl methacrylate. Specimens were cleared in oil of wintergreen, and transferred to containers of suitable sizes. A thick solution of the plastic in oil of wintergreen was poured over the specimens until each container was full. As soon as all bubbles had come to the surface of the solution and had been removed, each container was covered with a cap of the plastic. Specimens so prepared may be examined easily under the binocular microscope; visibility is high; there is no danger of leakage; there is excellent protection against damage.

### 141. A LOW VOLTAGE LAMP FOR GENERAL MICROSCOPIC USE. Elbert C. Cole, Williams College.

An improved model of the low voltage lamp shown at the Marine Biological Laboratory on August 31, 1938 has been made. The light source is an automobile headlight bulb, drawing its power from a variable transformer connected with the usual 110 volt A.C. circuit. Light is collected by a condenser and passes through a rod of the plastic, methyl methacrylate, which transmits but little heat. The rod carries a receptacle for the use of filters and a diffusing glass. This lamp has proved useful for the prolonged study of living tissues where heat must be largely eliminated. Excellent low power photomicrographs have been prepared. The cost of building, operating and replacement is small.

### 142. THE PLANKTON SAMPLER: A COMPACT DEVICE FOR INVESTIGATING QUANTITATIVELY THE DISTRIBTION OF ZOOPLANKTON. George L. Clarke, Harvard University.

The plankton sampler consists of a brass tube 5 inches in diameter and about 10 inches long to the rear end of which a conical net of any desired mesh may be attached by means of a bayonnette lock. At the front end of the tube a disc-shaped shutter is mounted and this is opened and closed by 'messengers' slid down the supporting cable.

Within the rear half of the tube a large propeller is mounted. This is geared to a counter of the cyclometer type which indicates the volume of water which has passed through the tube and net. The meter starts when the shutter opens and stops when it closes. It is therefore unnecessary to know the exact time when the messengers strike. Similarly an accurate knowledge of the boat's speed and of the extent to which the net has become clogged is unnecessary since the meter records only the volume of water which has actually passed through the net.

The plankton sampler, which weighs only 10 pounds, is operated on a light cable or rope and may be towed at any desired depth from either a rowboat or a power boat in either fresh or salt water. The instrument was designed primarily for catching effectively the copepod and cladoceran elements of the plankton and all smaller animals, but it has taken sagittae satisfactorily and may prove useful for other organisms.

142A. AN EXPERIMENTAL STUDY OF INSECT POPULATION. Royal N. Chapman, University of Hawaii.

Populations of the confused flour beetle, Tribolium confusum, have been carried on for nearly 6 years, during which time the entire population has been counted at regular intervals. Graphs showing the parallel progress of four populations are presented. Experimental populations have been set up to simulate the various long-time populations with various factors adjusted to determine their effect upon population trends. These illustrate the effects of varying egg laying rates and the competition of individuals within the population.

## PAPERS READ BY TITLE

143. THE DEVELOPMENT OF HABROBRACON EGGS IN DIFFERENT AQUEOUS MEDIA. Elias M. Spotkov, Washington Square College, New York University. (Introduced by Ruth B. Howland.)

Aqueous solutions have been found to permeate the chorion of the egg of Habrobracon. For experimental parthenogenetic studies now in progress, a suitable aqueous medium was sought in which Habrobracon ova may develop. In accordance, eggs were placed in the following media and their development noted by means of the microscope: distilled water, ethyl alcohol (50%), glycerine (50%), sea water, buffered amphibian Ringer's and Belar's solutions.

Eggs developed neither in distilled water, ethyl alcohol, nor clycerine due to the penetration of these substances through the chorion as evidenced by their effects on the ooplasm.

However, ova introduced into sea water or buffered amphibian Ringer's or Belar's solutions develop into normal larvae. The latter feed and grow when set on paralyzed moth (Ephestia) caterpillars, and metamorphose into apparently normal imagoes.

144. OBSERVATIONS ON THE KARYOKINESIS DURING CLEAVAGE OF THE EGG OF THE ZEBRA FISH (BRACHYDANIO RERIO). Edward C. Roosen-Runge, Brown University. (Introduced by J. W. Wilson.)

In the zebra fish egg the division of the living nuclei can be observed and many details, seen in fixed and sectioned material, can be confirmed or proved. The outstanding feature of karyokinesis in teleost blastomeres is the formation of chromosomal vesicles in the telophase. These vesicles persist through the interkinetic phases and break down in the prophase without the appearance of a distinct spireme stage. To this type of mitosis there is contrasted the common type of nuclear division in later development and in the adult. In this type no chromosomal vesicles appear in the telophase but a distinct spireme thread is always observed in the prophase. During the first eleven cleavages of the egg the first type of karyokinesis occurs, the second type begins to appear immediately afterward. This change in the mode of nuclear division coincides with the breakdown of the synchrony of cell division which prevails during cleavage of the teleost egg.

### 145. THE DEVELOPMENT OF THE HYPOPHYSIS CEREBRI IN CYPRINUS CARPIO. John Maxwell Robeson, Syracuse University. (Introduced by V. F. Lindeman.)

The fertilized eggs of Cyprinus carpio were placed in an aspirator at 22°C. At regular intervals eggs were fixed in Bouin's fluid. In this manner a complete age series was obtained. Up to the time of hatching, hourly age was used to show the stage of development, after hatching the length of the larva was used for this purpose. At hatching the larvae are from 4 to 5 mm. in length.

The first indication of the hypophysial anlage appears at approximately 75 hours after fertilization. This is a solid mass of cells proliferated between the anterior, ventral surface layer of the ectoderm and the base of the brain at the point where the infundibulum will appear. This cell mass is just in front of the anterior end of the notochord. The cells of the hypophysial primordium are loosely conformed, typical undifferentiated embryonic elements. These cells appear to be the same type as those composing the ectoderm beneath. At this stage the developing gland is closely adpressed to the floor of the embryonic brain. The hypophysial anlage is separated from the ectoderm beneath by a narrow space. No evidence of connective tissue or of vascular elements could be found at this stage. There is no hypophysial cavity since the gland does not develop from an invagination.

Nervous elements and blood vessels appear in the cell mass at the 7½-mm. stage and a definite connection with the brain is established.

In the 35-mm. carp the cells of the hypophysis show evidence of secretory products and the entire takes on the adult appearance.

### 146. PRODUCTION OF VARIOUS ABNORMALITIES IN AMPHIBIAN EMBRYOS BY CENTRIFUGAL FORCE. C. S. Thornton, Kenyon College. (Introduced by E. G. Butler.)

Eggs of Rana sylvatica and Amblystoma punctatum were centrifuged in the late blastula and early gastrula stages. One or sometimes two accessory tails, and anterior doubling of varying degrees were produced in the frog embryos. The accessory tails may occur at any point on the body, but are most common on the dorsal and ventral sides of the body and on either side of the blastopore in a dorsolateral position. Those secondary tails occurring on either side of the blastopore are the result of a spina bifida condition, each of the separated neural folds giving rise to a tail. The dorsally located secondary tails are formed from small segments of neural fold material which projected from the posterior region of the neural folds during the neurula stage. The origin of the ventrally located secondary tails is harder to determine. It is possible that these tails are produced from tail-forming material displaced by the centrifuging, for neural tube-like structures are found at the proximal ends of these ventral tails. Fifty per cent of the frog embryos showed anterior doubling of the neural folds. This doubling appears to be the result of a displacement of the walls of the blastocoel, an obstacle being formed on which the invaginating dorsal lip becomes split.

No accessory tails were produced in Amblystoma, but embryos with various abnormalities of the brain occurred. Embryos possessing simple tubular brains similar in structure to the posterior regions of the neural tube were common. These embryos never developed eyes, mouths, or other organs normally found on the head. Other embryos exhibited incomplete fusion of the extreme anterior part of the neural folds.

### 147. TEMPERATURE TOLERANCE AND RATES OF DEVELOPMENT OF THE EGGS OF AMPHIBIANS. John A. Moore, Columbia University. (Introduced by L. G. Barth.)

The temperature tolerance and rates of development of the eggs of Rana sylvatica, Rana pipiens, Rana palustris and Rana clamitans have been determined. Temperature tolerance of the eggs is correlated with the environmental temperature at the time of breeding. The eggs of Rana sylvatica, which are laid in March (dates are for New York City region) develop normally from 2°C. to 23°. Rana pipiens, which breeds about 2 weeks later, cannot tolerate a temperature of 2°. Normal development occurs between 8.4°C. and 26°; 30°C. is supramaximal. Rana palustris, the next species to spawn, develops normally between 8.6° and 30.4°C.; at 31.9° the embryos die. Rana clamitans lays its eggs in the late spring and early summer. Ten degrees centigrade is subminimal for development. Temperatures between 12.2° and 33.9° are suitable. At 36.1° the eggs die.

A marked difference was noted in the rates of development of these species. The eggs of the early breeding species develop more rapidly than those of the later breeding species. This observation has been extended to two additional genera, Amblystoma and Bufo. Amblystoma jeffersonianum and A. tigrinum breed before A. maculatum and embryonic development is more rapid in the former two. The earlier breeding Bufo americanus develops more rapidly than B. fowleri. In general the earlier breeding species are found farther north than the later breeding species.

### 148. REVERSAL OF POLARIZATION IN THE EAR PRIMORDIUM. Edmund K. Hall, University of Louisville.

Experiments were performed in which the ear primordium of Amblystoma punctatum embryos was transferred from the stage in which anteroposterior determination has just occurred (stage 21) to neural plate and fold stages, before anteroposterior determination. The orientation of the graft was such that only its anteroposterior axis was reversed with respect to that of the host. In one-third of the cases, ipsolateral ears developed, indicating that the determined anteroposterior polarization had been completely reversed. In another third of the cases, reduplicated ears developed, indicating partial reversal of the polarization.

In another series of experiments, the primordium was transferred from a stage in which dorsoventral determination has already occurred (stage 27) to a stage before dorsoventral determination (stage 21). The orientation of the graft was such that only its dorsoventral axis was reversed with respect to that of the host. In half of the cases, poorly differentiated ipsolateral ears developed, indicating reversal of the determined dorsoventral polarization. The remaining ears do not have criteria for laterality.

### 149. Some effects of colchicine on the early development of the frog, Rana pipiens. Ralph Judson Bushnell, University of Georgia.

Colchicine in solutions of 0.01 to 0.025 per cent markedly affects the developmental pattern of the frog when embryos are treated with it for 24 hours or longer. Stronger solutions can be used for shorter periods. The effect varies with the stage of development at which treatment is begun. Exogastrulae are produced by treating the earlier stages. Incomplete gastrulation usually follows the treatment of the blastulae. In such cases, the epidermis of the blastopore region is thickened and heavily pigmented, frequently exhibiting excessive proliferation which results in an epithelioma-like structure. Embryos treated in the yolk-plug stage are modified as follows: a marked shortening of the body with a ventral flexure of the tail; suppression of external gill development; bloating of the ventral body wall with some bloated areas on head and tail; suppression of endodermal differentiation; and, usually, a low grade of mesodermal differentiation. The duration of survival of treated embryos is directly correlated with the degree of modification as the result of cholchicine treatment.

### 150. Tympanic membrane induction in anuran integument. O. M. Helff, New York University.

Previous work by the writer has shown that normal development of tympanic membrane in R. pipiens is due to inductive influences of the annular tympanic cartilage. The present work concerns the nature of the development influences, specifically as regards whether or not non-living cartilage still possesses inductive properties. Experiments performed and results obtained were as follows:

Controls consisted of autoplastic transplantation of annular tympanic cartilage beneath back integument of metamorphosed R. palustris larvae. In general, integumentary thinning, coupled with partial to total glandular degeneration, occurred. This is typical of partial membrane formation.

Similar transplantations were made involving cartilage killed in ethyl alcohol, saturated chloretone solution, and boiling water or by desiccation in strong sunlight. The results differed considerably depending on type of killing treatment employed and degree of subsequent transplant degeneration. Partial membrane formation was usually induced, however, in the overlying back integument. Similar series were run with half-metamorphosed larvae. In general, membrane induction was even more pronounced.

The same experiments were performed on R. catesbeiana larvae. Transplantation, however, was made beneath skin posterior-ventral to the external nares Wide variation in results were observed. No membrane changes occurred in some cases, while in others considerable transformation was induced.

It is concluded that dead annular tympanic cartilage still retains inductive influences capable of initiating tympanic membrane formation in integument. These influences are, therefore, probably chemical in nature.

### 151. Development of tongue papillae in R. sylvatica. O. M. Helff and M. C. Mellicker, New York University.

The development of the tongue has been studied, histologically, in the adult, eleven metamorphic, and four premetamorphic stages of R. sylvatica. The main results recorded are as follows:

Four 'premetamorphic' papillae develop at an early larval growth stage. They form a row running crosswise of the dorsal-anterior region of the tongue anlage. Maximum size is reached during an early metamorphic stage, at which time they are long and slender with forked ends. Regression then occurs and they finally disappear shortly before metamorphosis is completed. Their significance is not known.

The origin, development, histology, and distribution of the filiform and fungiform papillae have been worked out. Points of difference observed were: Fungiform papillae arise from evaginations of the mucous epithelium at the onset of metamorphosis and possess connective tissue cores at all stages, while filiform papillae originate as groups of cells embedded in the tongue epithelium much later in metamorphosis and do not acquire fibrous cores until near the end of larval involution. Although filiform papillae consist entirely of non-ciliated columnar cells, the fungiform papillae include ciliated columnar, goblet, cylinder, and forked cells. In both types of papillae complete differentiation is attained by the end of larval involution; post-metamorphic changes consisting entirely of progressive increases in size.

The origin, development, and histology of the mucous glands and muscular elements have also been studied. Muscle fibers can be seen shortly after the onset of metamorphosis, while mucous glands develop at a rather late stage of larval involution.

### 152. Development of dermal plicae components of R. sylvatica. O. M. Helff and William Stark, New York University.

The development of dorsolateral and lateral R. sylvatica dermal plicae have been studied, histologically, in ten stages of larval involution and in the fully grown adult. The following points have been determined:

Shortly after metamorphosis begins, specialized cells with large nuclei are seen in the basal layer of the epithelium. These develop later into structurally similar glandular nests consisting of three to ten cells as seen in greatest cross section. Although the early structure of both mucous and poison glands is somewhat similar, it is concluded that they have separate origins. There is never a transformation later on of one type into the other.

Mucous glands develop in both dorsolateral and lateral folds at about the same time. Poison glands develop first in middle regions of dorsolateral folds. Lateral folds do not develop poison glands until near the close of larval involution. The mucous glands of both stripes and the poison glands of the dorsolateral folds are all fully differentiated by the end of metamorphosis. Considerable differentiation of poison glands in the lateral folds takes place, however, following larval involution.

Rudimentary poison granules probably arise from basal glandular cells which undergo disintegration. The granules are at first irregularly shaped and loosely constructed but become progressively more spherical and condensed.

The general histological changes in the epidermis, stratum spongiosum, and stratum compactum, as they occur during metamorphosis, have also been worked out.

### 153. GROWTH RATES OF THE HIND LIMBS OF RANA SYLVATICA DURING NORMAL AND INDUCED METAMORPHOSIS. Campbell M. Bower, McMaster University. (Introduced by A. Emerson Warren.)

Investigations of growth rates in the thigh, shank and foot regions of wood frog larvae have been made during the past several months. Careful measurements of 250 cases were taken at 3-day intervals. In half of the cases development proceeded normally. In the others metamorphosis was hastened by treatment with a 0.005% solution of di-iodotyrosin (Eastman Kodak preparation). Supplemental data have been obtained from some 950 cases which had been preserved at regular intervals for histological study.

Although in anurans the normal growth of the limbs with respect to the trunk is slightly heterogonic (Huxley), it has been found that within the limb itself the differential growth ratios of the thigh, shank, and foot remain constant only until the onset of prometamorphosis. With the rapid growth acceleration accompanying metamorphosis the percentage ratio of the foot with respect to the limb remains constant, while the corresponding ratios of the thigh and shank decrease and increase respectively. Thus during this period these limb components constitute an exception to the general principle of constant differential growth ratios. Moreover, there appear to be definite growth gradients within the appendage.

When the growth rate is further increased, as in cases of induced metamorphosis, the variation in growth ratios within the limb is not so evident.

There is no apparent difference between the growth of the limb during normal development and under conditions of precocious metamorphosis. Linear growth in each instance assumes the same general sigmoid curve. This agrees with observations made by Etkin for other species of Anura.

### 154. EFFECT OF A RESPIRATORY STIMULANT ON THE DEVELOPMENT OF THE TELEOST, ORYZIAS LATIPES. A. J. Waterman, Williams College.

For several years a study has been made of the effect of an accelerant of oxidative metabolism, 2, 4-dinitrophenol, on the development of the egg of the fresh water teleost, Oryzias latipes. Exposure was begun at four developmental stages: early and late cleavage, closure of the blastopore and optic vesicle. The most significant results appeared in concentrations of 1: 40,000 to 1: 160,000. Concentrations greater than 1:10,000 inhibited development and soon killed the embryo. The chorion exerts some protective action as shown by pricking before exposure is begun.

The toxic effect of this substance is confirmed. Retardation of development is proportional to the concentration and to the length of exposure. The effects are cumulative with time. Neither gastrulation, developmental rate nor hatching time are stimulated. Cell division may be retarded. Survival of the embryos is correlated with their sensitivity to the drug and sensitivity decreases in the more advanced stages. Temporary developmental inhibition may not necessarily cause irreversible injury, providing exposure is not too long. Abnormalities were particularly noticeable in the heart, extra-embryonic blood vessels, myotomes, tail, nervous system, eye, body size and proportion. No particular type of teratological embryo was produced but the effects on a particular stage are fairly consistent. Abnormal embryos do not hatch.

Gastrulation is either retarded, inhibited or goes on to completion depending on sensitivity. No exogastrulae were seen. Persistent blastulae and yolk-plug types may occur but do not develop. In many respects the results parallel those of Dawson for the frog embryo.

### 155. FORM REGULATION IN TRANSPLANTED EAR RUDIMENTS OF THE YOUNG CHICK EMBRYO. A. J. Waterman and H. J. Evans, Williams College.

Isolated ear rudiments of thirty-three 120-hour chick embryos and presumptive ear regions of earlier stages have been tested for morphological differentiation in chorioallantoic grafts. Graphic reconstructions and measurements, together with a few cardboard models of the better differentiated ears, have been made of all grafts obtained. Size and shape vary between grafts of the same stage, and all show abnormal form development. The cystic type is common. Grafts of older stages give more complete differentiation, but even these may show little resemblance to the control. One complete canal may be present together with the endolymphatic duct and sac, utricular and saccular regions, rudimentary lagena, and sensory regions. The sensory areas vary in number and size, while innervation and cupulae are often lacking. No trace of the horizontal canal is found but pouch-like evaginations of the epithelium, often in the vicinity of sensory areas, may represent the missing canals. The amount of cartilage varies and the labyrinth may be only partially inclosed. It is frequently difficult to identify the parts of even a good graft as histological differentiation may be retarded or malformed.

The chick inner ear rudiment generally does not give anything approaching a typical ear structure in chorioallantoic grafts. The potentialities are probably present but the environment is not favorable and the adjustment capacity shown by the rudiment is limited. These results closely resemble those secured in omental grafts of the rabbit inner ear rudiment (Waterman). Comparable shapes and degree of differentiation are obtained.

### 156. ON THE PRODUCTION OF FEATHER COLOR PATTERN BY MESODERMAL GRAFTS BETWEEN BARRED PLYMOUTH ROCK AND WHITE LEGHORN CHICK EMBRYOS. R. L. Watterson, University of Rochester. (Introduced by B. H. Willier.)

Implantation of small pieces of wing bud mesoderm from Barred Plymouth Rock donors (92 to 99 hours) into the right wing bud of White Leghorn host embryos (72 hours) produces black down feathers distributed over the entire wing and adjacent areas. A barred pattern very similar to that of control chicks of corresponding age appears in most juvenile feathers of these areas, but the total amount of pigment is distinctly less in these feathers than in controls. Since the only epidermal cells available for feather morphogenesis are those of host origin, these experiments show conclusively that the feather originates solely from host epidermis, and that the color pattern arises through the action of donor mesodermal cells (chromatophores).

Implants (mesoderm plus ectoderm) taken cephalad to Hensen's node or containing part of the node produce extensive areas of black down feathers, whereas material caudad to the node always yields negative results (donors 33 to 48 hours).

Somites and undivided segmental plate (72-hour donors) yield positive results, but neither wing nor leg bud mesoderm does so. Positive results are obtained from wing bud and somite mesoderm (92- to 99-hour donors); however, leg bud mesoderm gives negative results or pigment distribution limited to five to ten down feathers. These results show that pigment-forming cells are present in the node region and anterior to it in the segmental plate and somites of 33-hour to 72-hour donors; that between 72 and 92 hours pigment-forming cells also appear in the wing bud; that their appearance in the leg bud is somewhat later.

### 157. DEVELOPMENT OF EMBRYONIC GONAD GRAFTS IN JUVENILE DIAMOND-BACK TERRAPINS. Paul L. Risley, State University of Iowa.

This study represents an attempt to test the method of grafting as an experimental means of studying the problem of sex development in turtles, and to determine whether the differentiation of the morphologically bisexual gonads of embryonic stages can be influenced by their residence in juvenile hosts.

Grafts, consisting of a single mesonephros and gonad from donor embryos to 16 to 30 mm. carapace length, were placed on or near the gonads of 10-month-old juveniles. Ninety-nine operations were performed, and thirty-seven grafts were not recovered at autopsy after 37 and 120 days residence. Of 62 grafts recovered, 20 were degenerate and showed no recognizable gonad components. The remaining 42 contained germ cells in varying quantity: 13 showed germ cells present, but no sex cords or cortical tissues; 8 were rudimentary and bisexual, showing both sex cords and cortex; 21 were ovarian in structure, with cortical structures and ovocytes. Eighty-four hosts were females, and 15 were males.

The only evidence of male differentiation is seen in the rudimentary bisexual grafts, which occur only in female hosts. Failure to obtain male differentiation may be due to 1) chance selection of few male donor embryos and the extremely small number of male hosts; 2) inhibition of male development in grafts to female hosts; or 3) greater sensitivity of medullary tissues under the conditions imposed by grafting. If bisexual grafts are regarded as male, and all others female, the sex ratio (4.25 to 1) compares favorably with 5.66 to 1 host ratio, and indicates no sex reversal.

### 158. THE EMBRYOGENESIS OF DROSOPHILA FOLLOWING CENTRIFUGING AT HIGH FORCES. Herbert Shapiro, Princeton University and Clark University.

Although recent experiments involving centrifuging of organisms and embryos at high forces have demonstrated the possibility of at least temporary survival of these conditions, none have shown the complete survival of an embryo to the extent that a normal adult is ultimately produced. This has been found to be the case in experiments with embryos of the fruit fly, Drosophila melanogaster, whose eggs were centrifuged at forces varying from about 2000 (motor drive) to 200,000 × gravity (air turbine), i.e., up to centrifugal fields wherein organic and inorganic molecules may be displaced. The eggs were centrifuged at times from shortly after fertilization, to embryos several hours old. The per cent survival depends upon a) the embryonic stage at which centrifugation is carried out, and b) the duration of centrifuging. The quantitative details of survival cannot be given simply; however, it suffices for the present to state that in many cases normal imagos were produced, whose offspring, followed through several generations, were likewise normal. These experiments lead to the inference that if there is a molecular pattern or 'structure' (in the insect egg, at least) that either it is not necessary for development, or that it can be rearranged, or directed through a modified path, once its elements have suffered displacement.

### 159. THE TEMPERATURE EFFECTIVE PERIOD FOR WING LENGTH OF THE PENNANT WING OF DROSOPHILA MELANOGASTER. Fred Weinstein, Washington Square College, New York University. (Introduced by Morris H. Harnly.)

Cultures of homozygous pennant (vgP/vgP) were transferred at 6-hour intervals from 30° and 31°C. to 22°C. for completion of development. A sexual dimorphism was demonstrated since the female wings at all time intervals had a greater length than the male wings. The temperature effective period for wing length of males at 30° began between 96 and 102 hours and was completed between 156 to 168 hours from the time of egg laying; the temperature effective period for females being from 90 to 96 hours to 156 to 168 hours. For 31° the temperature effective period for wing length is the same for both males and females, starting at 96 to 102 hours and ending at 156 to 168 hours. Complete development at 31°C. was obtained for the first time with this stock; the number of adult flies appearing in ratio to the eggs laid being approximately 1: 200. The data for the temperature effective period for wing area are available but not completely tabulated. In agreement with previous workers, flies with normal wing margins did not appear in the controls having complete development at 22°, 30° and 31°C. However, wings with normal margins appeared for the first time at these temperatures in the transferred flies. The frequency of normal wing margins was greater in the females than in the males.

### 160. PARTHENOGENESIS IN TERMITES. S. F. Light, University of California.

In groups of unpigmented female nymphs of Zootermopsis angusticollis and Z. nevadensis isolated from functioning reproductives, one or more individuals develop into pigmented supplementary reproductives, as they would in isolated groups of mixed sexes. These isolated females lay eggs which hatch and develop into seemingly normal nymphs of both sexes. These isolated supplementary females are believed to be virgin, since unpigmented females from which they develop have never been known to copulate and have immature seminal vesicles in which spermatozoa have never been found. Furthermore, spermatozoa have not been found in the supplementary females developed in the absence of males, although they are conspicuously present in all ordinary female supplementaries examined. The female nymphs produced by these isolated females show the diploid chromosome number of 52; the number is not yet determined for the male.

The literature contains no report of the occurrence of parthenogenesis in Isoptera. In nature, sexually developed males are practically always present, and there is nothing to suggest anything unusual in reproduction. Indeed, now that the possibility of parthenogenesis is known, there is no information as to its role in the development of the complex termite colony.

### 161. THE EFFECTS OF METHENAMINE, ETHYL CARBAMATE AND POTASSIUM CYANIDE ON 'VITAL STAINING' OF THE CHROMATOPHORES OF THE SQUID. Elbert C. Cole, Williams College.

The optimum concentration of methylene blue for staining the chromatophores of the squid was found to be one part in 20,000. Of the various solvents tried, sea water was the best. The living squid was placed in a jar nearly full of staining solution, and the jar tightly sealed. An hour or two later, upon exposure to air, staining appeared irregular, but frequently selective.

The addition of 1% of methenamine to the staining solution before use increased selectivity for the nervous elements associated with the chromatophores. The addition of 0.3% of ethyl carbamate gave similar results. When potassium cyanide, 1 part in 200,000, together with 0.3% ethyl carbamate, was added to the staining solution, highly selective staining of the radial muscles of the chromatophores was secured.

Although selective permeability of the membranes of living cells was doubtless a factor in the penetration of the dye, the reoxidation of the reduced dye in the cells by atmospheric oxygen was definitely facilitated by the inhibition or destruction of the reducing mechanisms of the cells. The extent to which this staining may be called vital is not clear. Nerve fibers appeared so heavily stained that they could hardly be considered alive. Muscle cells faintly stained were seen to contract vigorously, but no contraction occurred in muscles whose nuclei had been distinctly stained. No evidence was seen to support the concept of a double innervation of the chromatophores.

### 162. A PHYSIOLOGICAL SOLUTION FOR THE LOBSTER HEART COMPARED TO ISOTONIC GLUCOSE, UREA AND SINGLE SALT SOLUTIONS. William H. Cole and Louis A. Kazalski, Rutgers University.

Based upon chemical analyses of mixed lobster serum, a physiological solution has been prepared, tested by perfusion on hearts of lobsters, Homarus americanus, and found satisfactory for normal beating of the heart for as long as 25 hours, using as criteria regularity of beat, amplitude and shape of kymographic records. Data for serum are as follows: $\Delta = 1.7495°$; osmotic pressure $= 21.05$; pH $= 7.44$; molarities: NaCl—0.43, KCl—0.014, $CaCl_2$—0.024, $MgCl_2$—0.004, $MgSO_4$—0.004, $NaHCO_3$—0.002; total molarity: 0.478. Since analyses did not include organic ions, since $NaHCO_3$ caused precipitation of $CaCO_3$ and $\Delta$ of the solution was only 1.6576°, the molarity was increased to 0.5 and the pH adjusted to 7.4 by NaOH. The successful solution, therefore, had the following molarities: NaCl—0.452, KCl—0.015, $CaCl_2$—0.025, $MgCl_2$—0.004, and $MgSO_4$—0.004.

By improved methods of exposing crustacean hearts and of recording, the complete solution was compared with isotonic solutions of single salts, urea, glucose and mixtures of salts containing varying ionic ratios. Adrenalin was tested. Diastolic arrest was caused by KCl, $CaCl_2$, $MgCl_2$, $MgSO_4$ and glucose; systolic arrest by NaCl, urea and by KCl and $CaCl_2$ mixtures in the above ratio. Adrenalin in small doses (1–10,000) caused no effect; in large doses (1–100) decreased frequency and amplitude. Lithium, bromine and iodine could not replace sodium and chlorine. Calcium ratio limits were narrow, potassium less so; magnesium and sulphate ions relatively unimportant; osmotic limits rather wide if ionic ratios correct; narrow if incorrect as in van't Hoff's solution.

### 163. RELATIONSHIP BETWEEN CYANIDE SENSITIVITY AND RATE OF RESPIRATION.

Wilbur A. Robbie, State University of Iowa. (Introduced by J. H. Bodine.)

The inhibition produced by cyanide and the time required for recovery after exposure may be used as indications of the cyanide sensitivity of the developing eggs of the grasshopper, Melanoplus differentialis. There is an apparent correlation between the sensitivity and the rate of respiration as evidenced by: 1) Eggs in $O_2$ have a higher respiration than those in air. When treated with CN they show a greater sensitivity than those exposed under comparable conditions in air and $N_2$. Similarly, eggs in manometers that are shaken rapidly respire at a greater rate than those in which the experimental fluid is stationary; they also show a correspondingly increased sensitivity. 2) The normal rate of respiration rises with increase in temperature, and the sensitivity to CN appears to vary in the same manner. Eggs exposed at 10°C. are only slightly affected by doses of CN that produce 85% inhibition at 30°C.

### 164. RESPIRATION OF ASTASIA SP. AND KHAWKINEA HALLI.

George E. Jay, Jr., State University of Iowa. (Introduced by T. L. Jahn.)

The respiration of Astasia sp. and Khawkinea halli was measured in bacteria-free cultures by means of small differential manometers. The immersion fluid was diluted Belar solution buffered with phosphate to pH 7.0. Astasia consumed about 24 mm.$^3$ of oxygen per hour per 10,000 organisms with a respiratory quotient of 0.36 (0.30 to 0.43). The oxygen consumption of Astasia was decreased 61% by the addition of 0.01 M KCN. Khawkinea halli consumed about 20.5 mm.$^3$ $O_2$ per hour per 10,000 organisms with a respiratory quotient of 0.56 (0.46 to 0.67). The oxygen consumption of Khawkinea was depressed 64% by 0.01 M KCN.

The low respiratory quotients of these organisms indicate either the conversion of protein to carbohydrate or the incomplete oxidation of carbohydrate.

### 165. THE EFFECTS OF HOMOLOGOUS CARBAMATES ON THE RESPIRATION OF BLOCKED AND ACTIVE EMBRYOS.

Guy M. Everett, State University of Iowa. (Introduced by J. H. Bodine.)

The effects of ethyl, n-propyl, and n-butyl carbamate on the respiration of blocked (diapause) and active (postdiapause) grasshopper embryos have been studied.

The oxygen consumption of active embryos is depressed approximately 50% (to the diapause level) by 0.25 M ethyl carbamate or 0.025 M n-butyl carbamate. The blocked embryo is depressed only 10%.

The rates of oxygen consumption expressed as millimeters$^3$ of oxygen per embryo per hour for morphologically similar blocked and active embryos show that concentrations up to 0.25 M n-butyl carbamate have a slight stimulating effect on diapause respiration but reduce the respiration of the active embryo to the diapause level. In concentrations of 0.025 M and higher, both active and blocked embryos are depressed to the same rate of oxygen consumption.

The reversible concentrations of carbamates are antagonized completely by $12.5 \times 10^{-5}$ M methylene blue, the oxygen uptake of narcotized postdiapause embryos being restored to its normal level, and the narcotized diapause embryo being stimulated to the postdiapause level.

The dinitrophenol stimulated respiration of both active and blocked embryos is antagonized by the carbamates.

### 166. THE ELECTRICAL RESPONSE OF THE COMPOUND EYE OF THE CECROPIA MOTH.

Theodore Louis Jahn and Frederick Crescitelli, State University of Iowa.

The electrical response of the dark-adapted eye of Samia cecropia to brief flashes of white light consists of a series of waves including an initial rapid positive (with reference to the illuminated eye) a-wave; a second, rapid negative b-wave; and a third, very prolonged c-wave. Light adaptation decreases the magnitude of the electric response and alters its form. With light adaptation the a-wave disappears, the b-wave is decreased, and the c-wave is markedly reduced in magnitude and duration. With dark adaptation the original wave form is gradually restored, the restoration taking place over the period of at least an hour. Minute oscillations are also present in the response of the moth eye to white light, but no wave comparable to the d-wave of the grasshopper or vertebrate response has ever been recorded.

When the eye is illuminated with light of different spectral composition but of equal energy content, the magnitude of the c-wave varies according to the quality of the stimulating light. The magnitude is greatest with green light and declines with blue, violet, orange-red and red illumination. As in the grasshopper eye, there appears to be in the moth eye no specific effect of wave length per se on the wave form of the electric response.

### 167. ABSORPTION OF LIGHT IN THE OKOBOJI LAKES.

A. B. Taylor and Theodore Louis Jahn, University of Illinois and State University of Iowa.

In August, 1938, absorption of light was measured at various depths in the Okoboji Lakes by means of a photronic cell and a tungsten filament lamp which were mounted behind glass windows in separate metal housings so that a parallel beam of light passed through ½ meter of water before striking the photronic cell. This apparatus could be lowered to any desired depth, and the transmission at that depth could be measured with a microammeter. This was converted to percentage transmission through 1 meter by comparison with a similar reading taken with the apparatus in air.

After the formation of the thermocline in Lake West Okoboji the transmission per meter was about 30% for the upper 10 meters, rose sharply between 12 and 13 meters to 46%, remained about constant to 17 meters, rose sharply to 74% at 18 meters and then gradually to 81% between 22 and 28 meters. Below 28 meters the transmission dropped to about 70%. These data are being studied in relation to the distribution of plankton.

The average transmission of Lake East Okoboji was about 0.0002% per meter and varied only slightly with depth.

### 168. THE ELECTRICAL RESPONSE OF THE GRASSHOPPER EYE TO COLORED LIGHT.

Frederick Crescitelli and Theodore Louis Jahn, State University of Iowa.

The electrical response of the dark-adapted grasshopper eye to brief flashes of white and of different colored lights varies according to the intensity of the stimulating light. At very low intensities the response consists of an initial positive (with reference to the illuminated eye) wave, resembling the a-wave of the vertebrate electroretinogram, and a secondary negative deflection. As the intensity is increased the relative magnitude of the positive wave decreases while that of the negative wave increases. At high intensities the positive wave is entirely absent and the response consists only of the apparently monophasic negative phase.

The electrical response to different colors possesses all the waves (a, b, c and d) which are characteristic of the response to white light. The same conditions that are necessary to elicit these waves with white light are essential in the case of colored light. As far as can be determined, no specific effect of wave length per se is evident.

When the eye is illuminated with different colored lights of equal energy content, the magnitude of the monophasic phase varies according to the spectral composition of the light. The magnitude, greatest with green light, decreases respectively with blue, violet, orange-red and red illumination. The curve relating the magnitude of the response to wave length has a general similarity to the absorption curve of visual purple.

### 169. THE RELATION OF THE NERVOUS SYSTEM TO SKIN POTENTIAL IN THE INTACT FROG. F. R. Steggerda and E. Ponder, University of Illinois and Cold Spring Harbor.

By making a small opening in the skin on the ventral surface of the frog, electrodes were adjusted to record E. M. F. potentiometrically. When readings were taken at 3-minute intervals, no appreciable fluctuation in potential was noticed 10 minutes after the start of the experiment. After single pithing, a definite drop of approximately 25% in potential was noted, with an additional decrease of 25% following double pithing. These changes were sudden and showed very little alteration in the 15-minute interval, following the operation. Following this, when the skin was removed, there was another similar decrease in potential, which continued to fall progressively for approximately 45 minutes, after which an equilibrium was established.

Experiments in which the ventral skin had been previously denervated showed a decrease similar to that of the pithed animal. Strychnine injections increased the potential about 30% above normal, within 9 to 12 minutes, after which there was a gradual return to normal. Removal of the heart had very little immediate influence on the skin potential. It appears that skin potential is related to a tonic influence of the central nervous system.

### 170. A STUDY OF THE VITAMIN E DEFICIENCY EFFECTS IN MALE AND FEMALE RATS REARED ON AN IRON TREATED RATION. F. B. Adamstone and Edna M. Pratt, University of Illinois.

A careful histological study has been carried out on the testes of male rats reared from weaning on a ration treated with ferric chloride to produce vitamin E deficiency in comparison with the effects of using a synthetic E-deficient ration. It has been found that a sequence of degenerative changes occur which are identical to those observed after use of a synthetic ration, namely: fusion of sperm heads, beading and chromolysis of the nuclei of spermatids, giant cell formation in spermatocytes and sloughing of germinal epithelium. Curative experiments carried out with semi-castrate rats showed that the remaining testis was injured beyond repair even though vitamin E concentrates were provided after 10 months on the treated ration.

In female similarly reared, matings were made beginning the sixth month but these were sterile. After supplying vitamin E concentrates positive matings were secured but the reproductive performance of all females was poor and never returned to normal.

### 171. THE HYDROGEN-ION CONCENTRATION IN THE DIGESTIVE TRACT OF PERCH. Bertil G. Anderson, Western Reserve University.

Perch caught in nets were held in aquaria without feeding. At various intervals they were killed and the gut contents examined. The hydrogen-ion concentrations were determined by means of a glass electrode. For the most part the contents of the gut consisted of Cladocera. Individuals whose stomachs were still filled when the examinations were made showed an average hydrogen-ion concentration of pH 3.5 for the stomach and a slightly alkaline condition for the intestine. The alkalinity of the intestinal contents usually increased as the posterior-end was approached when the contents showed a pH 8 to 8.5. As the alimentary canal emptied the reaction of the lumen approached neutrality.

### 172. THE RESPONSES OF THE SWIMBLADDER OF THE GUPPY, LEBISTES RETICULATUS, TO SUDDEN DECREASES IN PRESSURE. Frank A. Brown, Jr., Northwestern University.

The guppy, Lebistes reticulatus, is unable to adapt its swimbladder to pressure decreases of more than 120 mm. Hg below atmospheric pressure when the water remains in gaseous equilibrium with air at the original pressure. Instead, the swimbladder increases in size (a response opposite to that of adapting the fish to the new pressure) at a rate which is a function of the pressure decrease. The explanation of this response is shown to be found in a reversal of direction of the normal concentration gradient for the gases. Normally, the gradient favors outward diffusion. The guppies, furthermore, during 2 to 5 hours following the pressure decrease, are able to oppose more or less successfully the inward diffusion of gases to the bladder. Here again, certain evidence suggests quite strongly that this initial and transitory attempt at adaptation is explainable wholly in terms of physical diffusion of $CO_2$ which is present in higher percentage in the bladder than in air. Thus, guppies appear to be dependent solely upon physical diffusion for removal of gases from the bladder. The high percentage of $CO_2$ in the gas secreted into the bladder plays an important role.

### 173. CHANGE IN THE COAGULATION TIME OF BLOOD OF GALLERIA MELLONELLA DURING METAMORPHOSIS. Ivon R. Taylor and Nathan Millman, Brown University.

The time necessary for the coagulation of the blood of Galleria mellonella in the late larval stages and early pupal period was measured by means of a Rieker coagulometer. Individual samples of blood from 188 animals were tested, a single drop being used for each measurement. In the late larval stages, the animals investigated were designated and grouped as toppers, young spinners, middle-aged spinners and old spinners. In the early pupal stage, the animals were used within 14 hours after pupation. The various stages are mentioned above in the order of progressive development and each group consisted of more than thirty animals. An interval of about 24 hours separates the beginning of one stage from that of the next at 30°C., the temperature at which the animals were raised. The average coagulation time of the blood of the old spinners was found to be almost twice as long as that for the other groups, the difference being statistically significant. Some blood samples showed no coagulation within 30 minutes and such samples were regarded as non-coagulable. The percentage of cases exhibiting non-coagulability was greatest for the old spinners but no such cases were found among the toppers, the youngest larvae used. It is concluded that in the final stage of larval life, just before pupation, the blood shows a decrease in its ability to coagulate.

### 174. THE THICKNESS OF THE LIMITING ENVELOPE OF THE RABBIT ERYTHROCYTE. Francis O. Schmitt and David F. Waugh, Washington University.

Polarization optical experiments have demonstrated that the lipide and protein molecules in the rabbit red cell membrane are oriented in a manner similar to that of built-up mixed films of stearate and protein molecules. The refractive index of the red cell membrane is also similar to that of stearate built-up films. To determine the thickness of the membrane it is, therefore, only necessary to deposit the washed red cells on a slide, remove the haemoglobin by lysis (osmotic or surface active), dry, and match the color and intensity of the double membranes against standard step films of stearate (the step thicknesses of which are known). This is done with illumination at normal incidence, the glass slide being of high refractive index and painted on the under side to prevent reflection of light from this surface.

Values for the membrane thickness of cells haemolysed in distilled water average about 160 AU (0.016 $\mu$). Leaching with lipide solvents reduces the apparent thickness by approximately 80 to 100 AU, the thickness of the protein being therefore approximately 60 to 80 AU. The apparent thickness also depends markedly on pH, being somewhat greater after haemolysis in acid buffer (pH 5 to 6) and much less after haemolysis in dilute alkaline buffer (pH 9 to 10). The method therefore gives information of an analytical sort and the results have immediate bearing on the question of the structure of the membrane and the mechanism of permeability processes.

### 175. HYDROGEN-ION CONCENTRATION OF INSECT BLOOD. Robert D. Boche and John B. Buck, Carnegie Institution of Washington, Baltimore.

The H-ion concentration of the larval blood (haemolymph) was determined in Drosophila, Sciara and Chironomus, using 1) colorimetric indicators (C), 2) microquinhydrone electrodes (Q.E.), 3) capillary glass electrodes (G.E.). The pH of Drosophila blood average 7.1 (C), 7.12 (Q.E.), 7.10 (G.E.); that of Sciara 7.1 (C), 7.37 (Q.E.), 7.12 (G.E.); that of Chironomus 7.56 (Q.E.), 7.51 (G.E.). The H-ion concentration of the blood of a given lot of larvae is ordinarily very close, but different lots of larvae may differ somewhat from each other.

If Chironomus blood stands exposed to air its pH value (G.E.) increases steadily at the rate of 0.2 units per ½ hour. We attribute this increase in alkalinity to escape of $CO_2$ from blood buffers. If, however, the bled larvae are left in their blood, it grows steadily more acid, decreasing about 0.2 pH units in ½ hour, due presumably to accumulation of acid metabolites from the tissues. Blood covered with a thin layer (1 mm.) of paraffin oil grows alkaline, but more slowly than exposed blood. Five millimeters of paraffin oil, however, maintains the H-ion concentration nearly constant.

In both Sciara and Chironomus certain regions of the gut have a pH (C) remarkably high (8.5) and other regions low (5).

The results are regarded as indicating a need for great care in buffering solutions used to examine normal 'living' insect tissues in vitro.

### 176. The nature of the relation between the amount of closure and the size of the wound in the carapace of Daphnia. Bertil G. Anderson, Western Reserve University.

The amount of closure of a wound during any instar increases with the size of the wound up to a maximum after which it decreases when wounds of the greatest possible size range are secured. The amount of closure appears to be a function of the perimeter of the wound in as much as the amount of closure per unit perimeter is relatively constant over two-thirds the possible size range. The amount of closure per unit perimeter increases to a constant value as the size of the wound increases at the lower end of the size range. In the maximum size range, the amount of closure per unit perimeter decreases as the size of the wound increases.

### 177. Occurrence of mitosis in diapause and postdiapause Melanoplus differentialis embryos. Theodore N. Tahmisian, State University of Iowa. (Introduced by J. H. Bodine.)

This study was undertaken in an attempt to determine whether mitosis occurs in the Melanoplus differentialis embryos which are subjected to 5°C. after they have entered diapause.

Embryos which had entered diapause were kept at 5°C. After 3 months, some embryos were dissected and fixed. Other embryos were incubated at 25°C., and at hourly intervals some embryos were dissected and fixed. A second group that had been kept at 5°C. for 9 months was treated in like manner. In either case, embryos that were kept at 5°C. showed no mitotic activity.

Embryos that were kept at 5°C. for 3 months showed mitotic figures 14 hours after they were incubated at 25°C., and those that were kept 9 months at 5°C. showed mitotic figures 11 hours after they were incubated at 25°C. After 24 hours incubation at 25°C., the mitotic activity of the embryos that had been subjected to 5°C. for 9 months was three times as great as that of embryos that had been subjected to 5°C. for 3 months.

Mitotic activity of the diapause and postdiapause Melanoplus differentialis embryos correlates closely with the oxygen consumption rates and other physiological phenomena as found in this laboratory.

178. BACTERICIDAL ACTIVITY OF 'ROYAL JELLY' OF THE HONEYBEE. C. S. McCleskey and R. M. Melampy, Louisiana State University and Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

'Royal jelly,' the secretion of the pharyngeal glands of the worker honeybee which is the food of the queen, has pronounced bacteriostatic and bactericidal properties. Escherichia coli and Eberthella typhosa are inhibited by a concentration of 1 to 50 of the natural jelly in nutrient broth; Staphylococcus aureus and Bacillus metiens by a concentration of 1 to 100. The germicidal activity is markedly influenced by temperature. When 1 cc. of broth culture was added to 1 cc. of a one-fifth dilution of 'royal jelly' at 43° to 45°C., the organisms were destroyed in 15 seconds; at 23° to 25°C., in from 10 to 30 minutes; while at 5°C. the organism survived for 2 days. The bactericidal potency was rapidly reduced by neutralization of the natural acidity of pH 4.4 to 4.6. Sterilization of test cultures required only a few minutes at pH 4.6 (25°C.), but required 2 days at pH 7.0. The bactericidal capacity was largely lost when 'royal jelly' was filtered through paper; it was found in the sediment after centrifugation. The active principle was removed from 'royal jelly' by extraction with ethyl alcohol and with acetone. From these extracts, crystals were obtained which seemed to possess the bactericidal principle.

179. THE RELATION OF IMAGINAL POPULATION DENSITY TO THE POST-EMBRYONIC DEVELOPMENT OF TRIBOLIUM CONFUSUM LARVAE. Thomas Park, Ella Virginia Miller and Catherine Z. Lutherman, The University of Chicago.

A series of 45 larval Tribolium populations was set up each containing 10 larvae in 8 gm. of flour. Imago beetles were introduced into these cultures to form the following experimental series: I, 10 larval bottles each containing 1 imago; II, 10 larval bottles each containing 4 imagoes; III, 10 bottles with 16 imagoes each; IV, 10 bottles with 32 imagoes each and, V, 5 bottles with 64 imagoes each. It was shown that as the imago density increased the duration of the larval period lengthened and the larvae grew less in terms of body weight.

180. PIMELIC ACID AS A GROWTH FACTOR FOR THE CILIATE, COLPIDIUM CAMPYLUM. R. P. Hall, New York University.

A gelatin-dextrose medium containing pimelic acid (Eastman preparation) in a concentration of $10^{-6}$ gm. per cubic centimeter has supported growth of bacteria-free strain of Colpidium campylum through six successive transfers over a period of more than 2 months. In the control medium without pimelic acid the ciliates grew only in the first and second transfers. Similarly, the strain has been carried through five transfers in a gelatin medium containing pimelic acid, while the control medium failed to support growth beyond the first transfer. It thus appears that the failure of the ciliates to grow in gelatin is not dependent necessarily upon the amino-acid deficiencies of gelatin. The implication is either that the missing amino acids are not essential to life of the ciliates or else that synthesis is possible in the presence of pimelic acid.

Similar investigations with suberic, adipic, azelaic, sebacic, glutaric, succinic and oxalic acids are in progress. Some of these series are in the second transfer and others in the third. Certain of these acids appear to resemble pimelic in relation to growth, but the results are not yet conclusive and will be reported in detail later.

### 181. THE ORIGIN AND THE FUNCTION OF THE ACID AND THE ALKALI IN THE FOOD VACUOLES IN PARAMECIUM AND AMOEBA. S. O. Mast, Johns Hopkins University.

Paramecia and amoebae live and feed indefinitely in culture fluids which contain neutral red, Nile blue sulfate or phenol red in low concentration. If living organisms are ingested they soon die and then immediately become pink in neutral red and blue in Nile blue sulfate, i.e., acid, but the solution in the food vacuoles remains colorless. If starch is ingested it also remains colorless.

Paramecium contains numerous small droplets which become red in neutral red and blue in Nile blue sulfate. These droplets contain fatty acid. They aggregate on the surface of forming food vacuoles, remain for some time after the vacuoles have left the gullet and then disappear. They do not enter the food vacuoles.

The acid in the food vacuoles originates in the food in them, not in the cytoplasm around them. It does not kill the organisms and it probably has no function. Some time after the food vacuoles have left the gullet the entire content of those which contain organisms becomes yellow in neutral red, colorless in Nile blue sulfate and purple in phenol red (i.e., alkaline). The solution changes color first and then the organisms. Digestion begins immediately after the content of the vacuoles has become alkaline.

The alkali in the food vacuoles originates in the cytoplasm around them. It probably functions in digestion.

### 182. EXPERIMENTAL STUDIES ON THE LONGEVITY OF UNFERTILIZED EGG CELLS OF MACTRA SOLIDISSIMA. Victor Schechter, College of the City of New York.

The life span of the egg of Mactra in sea water is marked by a series of definite structural changes. Oval on emergence, the egg rounds in an hour or 2 and then gradually becomes indented as if subject to severe exosmosis. The germinal vesicle may persist for 12 to 24 hours. By decreasing calcium to one-sixth the normal amount the germinal vesicle may be preserved for as long as 95 hours; if in addition magnesium is tripled about 75% retained the vesicle for 150 hours. Germinal vesicle breakdown occurs within 10 minutes after insemination during the natural or extended life of the egg. It is believed that calcium and magnesium are here partly complementary in action on the vesicle, but magnesium seems to have a greater effect in preserving the spherical form.

The life of these eggs is also prolonged in pasteurized or Berkefeld filtered sea water. In sea water in which other eggs have disintegrated fresh eggs die quickly. The toxicity may be removed by pasteurization or Berkefeld filtration. It is not yet clear whether these agents act by removing living pathogenic bacteria or the products of decay. Counts of bacteria in the salt solutions which are effective in prolonging life revealed no difference in numbers which can be correlated with the action of ions on longevity.

### 183. DIFFERENCES IN THE PHYSICAL NATURE OF THE PROTOPLASM OF THE NEREIS EGG FOLLOWING ACTIVATION. Donald P. Costello, University of North Carolina.

It is well known that unfertilized and fertilized eggs of Nereis limbata differ in the physical nature of their protoplasm. Their behavior when subjected to high centrifugal force provides further evidence of such differences. By 60 minutes of centrifuging at 66,000 times gravity in isopycnotic sucrose the unactivated egg is elongated into a dumb-bell form but not fragmented, the activated egg is readily fragmented. The unactivated egg may be cytolysed by a higher centrifugal force or a longer period of centrifuging, but it never separates into two clean-cut fragments with the forces thus far applied. The activated egg, with appropriate forces, separates into two spherical fragments, the centripetal fragment containing mostly oil and hyaline protoplasm, the centrifugal fragment containing the yolk spheres, small basophilic granules, and a second type of hyaline material.

Eggs activated parthenogenetically in the centrifuge show the typical fragmentation of eggs inseminated or treated with parthenogenetic agents before centrifuging.

Fragmentability is undoubtedly the result of a complex of factors, among which the cohesive properties of the protoplasmic surface may be of importance. It is possible that a relationship exists between the fragmentability and the cleavage capacity of the protoplasm.

### 184. THE BEHAVIOR OF THE OIL DROPS IN THE CENTRIFUGED EGG OF NEREIS LIMBATA.

Donald P. Costello, University of North Carolina.

In the normal, non-centrifuged egg of Nereis limbata the double row of about thirty large oil droplets surrounding the germinal vesicle of the oocyte slowly coalesce during early cleavage until their number is reduced to four, one located in each of the four entomeres. Centrifuging the unfertilized egg at 66,000 times gravity for 60 minutes crowds the oil droplets into close proximity at the centripetal pole, but in the unactivated egg the oil droplets remain discrete until cytolysis ensues. If unfertilized centrifuged eggs are activated, either with sperm or with parthenogenetic agents, the oil droplets begin slowly to coalesce, and may form a single large drop. However, the oil droplets may be separated into groups by cleavage planes or by flowing movements of the protoplasm, in which case coalescence results in the production of more than one large oil drop. J. Spek has emphasized the alteration in the plasma colloids of the egg of Nereis dumerilii following activation which produces, among other results, the coalescence of the oil droplets.

In activated eggs, centrifuging accelerates coalescence so that one large oil drop is usually formed. In the two-cell stage centrifuging yields two coalescence drops, one in each blastomere. The centrifuging brings the oil droplets into contact much more rapidly than do the flowing movements of the protoplasm during the early cleavage. It is concluded that a change occurs at the protoplasm-oil interface upon activation which persists during early cleavage, permitting the coalescence of oil droplets which are brought into contact.

### 185. SURFACE CHANGES IN THE ARBACIA EGG UPON FERTILIZATION.

Robert Chambers, Washington Square College, New York University.

There is evidence in the literature that the unfertilized Arbacia egg is covered with a delicate vitelline membrane which, upon fertilization, becomes detached from the egg's surface and progressively hardens to form the fertilization membrane. The weakness of the vitelline membrane is such that an unfertilized egg with the membrane present readily breaks into two parts which immediately round up when a horizontally placed microneedle is lowered through the egg lying on the sea water/air interface of a hanging drop. As soon as the membrane becomes separated upon fertilization (either by sperm insemination or artificial activation) the membrane is too stiff to permit the above operation.

The egg within its fertilization membrane can be readily cut in two by pressing a horizontal microneedle into the egg against the cover slip if the operation is performed during the first 3 minutes after sperm insemination. Upon removal of the needle the still delicate fertilization membrane recedes with the needle and the hemisected egg appears as two rounded bodies more or less closely appressed within an intact fertilization membrane. The operation becomes increasingly difficult 4 minutes and later after insemination until, at 5 or 6 minutes and thereafter it becomes impossible. The deep compression produced by the needle may or may not disappear as the fertilization process proceeds.

The progressive increase in stiffness of the surface of the egg within its fertilization membrane coincides with the development of the hyaline plasma layer or membrane. Other evidence for the early appearance of the hyaline plasma layer is presented by Kopac.

### 186. EFFECT OF THE HYALINE PLASMA LAYER ON COALESCENCE OF ARBACIA EGGS WITH OIL DROPS. M. J. Kopac, Washington Square College, New York University.

Kopac and Chambers have measured the effect of the vitelline membrane on the coalescence of Arbacia eggs with oil drops. Fertilized eggs (in sea water about 2 minutes) with their fertilization membranes removed show over a tenfold increase in coalescency when compared with unfertilized eggs which still have the vitelline membrane. However, the coalescencies of fertilized eggs if kept in sea water decrease steadily and become zero after 15 or more minutes. The hyaline plasma layer is fully formed at this time.

Eggs with the hyaline plasma layer fully formed and then immersed in KCl solution for 10 minutes have a coalescency comparable to freshly fertilized eggs. After 20 to 30 minutes' immersion, the coalescencies are over 100 times higher than in unfertilized eggs. Eggs placed in KCl solution before the layer is formed and immersed for 10 minutes show coalescencies 50 times higher than unfertilized eggs. After 30 minutes' immersion, the coalescencies of these eggs are about 140 times higher than in unfertilized eggs, while freshly fertilized eggs are slightly more than 10 times higher.

The fact that fertilized eggs immersed in KCl solution have higher coalescencies than freshly fertilized eggs immersed in sea water suggests that the secretion of hyaline substance starts very soon after fertilization. Other evidence for the early appearance of the hyaline plasma layer is presented by Chambers.

In conclusion, Arbacia eggs with fertilization membranes removed and immersed in KCl solution for 30 minutes show the highest coalescencies indicating that these eggs have the minimum of extraneous coatings. It is entirely probable that the external surface on these eggs is actually the protoplasmic surface layer.

### 187. FURTHER STUDIES ON THE DEVAUX EFFECT AT OIL PROTOPLASM INTERFACES. M. J. Kopac, Washington Square College, New York University.

Every oil drop (oleum percomorphum) microinjected into Asterias oocytes (in sea water) produces the Devaux effect (wrinkling at oil surface) providing the cells are cytolyzed not more than 30 seconds later. The same reaction occurs on oil drops introduced within 15 seconds after cytolysis. Also 99% of the oil drops microinjected into oocytes in sea water within 1 minute before cytolysis and 50%, if introduced within 1 minute after cytolysis, give the Devaux effect.

In other solutions, the percentages of oil drops introduced into oocytes within 1 minute before or after cytolysis which give the Devaux effect are as follows: a) isosmotic NaCl, KCl, $CaCl_2$ or $MgCl_2$, 90 and 20%, respectively; b) 0.33 or 0.50 M urea in sea water, 90 and 0%; c) 0.67 M urea in sea water, 0 and 0%. The latter result is of interest since protein molecules are known to dissociate in strong urea solutions.

About 90% of the oil drops in contact with cytoplasm for 4 minutes fail to give the Devaux effect on cytolysis if the oocytes are in sea water. Yet, every oil drop in contact with cytoplasm for the same period becomes wrinkled on cytolysis if the oocytes are in NaCl or KCl solutions.

The failure to obtain the Devaux effect on oil drops which were in contact with cytoplasm several minutes before cytolysis or in oocytes immersed in 0.67 M urea may be due to changes in tension occurring at the oil-protoplasm interface. The adsorption and spreading forces of proteins are conditioned by the tension at the interface. Final interpretations must be reserved until complete data are available on the effects of single salts, urea, and time on oil-protoplasm interfacial tensions.

### 188. THE CHEMOTACTIC RESPONSE OF THE POLYMORPHONUCLEAR GRANULOCYTES TO BACTERIAL COLONIES. C. G. Grand, Washington Square College, New York University. (Introduced by M. J. Kopac.)

Mouse embryonic spleen was grown in tissue culture in media inoculated with Staphylococcus aureus. Within a few hours of incubation the granulocytes migrated in clouds from the explant. During this time the bacteria developed into colonies beyond the zone of outwandering granulocytes. Some of the colonies were smooth, round and homogeneous in appearance while others, equally distributed, were irregular in outline and appeared roughly granular.

Some time later the granulocytes could be seen wandering between the bacterial colonies. It was noted that the granulocytes were definitely attracted to the rough colonies which they penetrated in large numbers. These colonies were soon destroyed. Many of the granulocytes invading these colonies died while others left the region and migrated toward other colonies. On the other hand, granulocytes were observed to come into contact with the surface of a smooth colony and to move along it without being attracted.

An interesting phenomenon was observed regarding the fate of the smooth colonies. On the side of a smooth colony toward a crowded mass of granulocytes occurred a localized, granular break down of the smooth surface. Thereupon, the granulocytes in the vicinity turned in their paths and advanced directly toward the roughening portion of the colony which they penetrated. The attraction continued until the entire colony roughened and finally was destroyed. There appears to be a substance which diffuses from a mass of living granulocytes and causes the smooth surface of a round colony to break down and liberate a granulocyte-attracting agent.

### 189. A CINEMATOGRAPHIC METHOD OF STUDYING DISPERSION AND CONCENTRATION OF CHROMATOPHORIC PIGMENT. Earle B. Perkins and William H. Cole, Rutgers University.

Quantitative accounts of dispersion and concentration of chromatophoric pigment in animals responding to color of background demand reproducible accurate measurements of pigment volume or area at short consecutive intervals during a color change. A cinematographic method has been developed which is preferable to other methods commonly used. It consists in photographing a given area of a living animal during a color change, by means of an electrically controlled mechanism which accurately operates the camera for any desired time lapse and exposure. The animal is not injured thereby and tests may be made on the same individual repeatedly. The ciné-negatives provide a permanent record of individual chromatophores and an opportunity to study their shape, size, color and behavior.

By the method, an area (1.4 × 2.0 mm.) on the back of the head of Fundulus heteroclitus, in fresh water, has been studied during successive changes of the background from white to black and vice versa. From the enlarged prints, measurements of area of individual melanophores in the superficial layer have been made for several background changes. Preliminary analysis of the data indicates that 1) plots of percentage area increase and decrease are smooth curves essentially like those reported by Hill, Parkinson and Solandt; 2) rates of dispersion and concentration of individual melanophores may vary slightly during successive color changes; 3) 'pulsation' of melanophores sometimes occurs after the period of maximum change; 4) the mechanism of concentration is not merely the reverse of dispersion.

### 190. THE EFFECT OF TEMPERATURE ON THE SIZE OF THE COLUMNAR CELLS OF THE MID-INTESTINE OF JAPANESE BEETLE LARVAE. Daniel Ludwig, University College, New York University.

Larvae of the Japanese beetle, reared at temperatures of 20° and 25°C., were fixed at different stages of development and a study was made of the number and size of the columnar cells which occur in the region of the mid-intestine limited by the second and third rings of gastric caeca.

Larvae were heavier when reared at the higher temperature, the freshly molted second instars averaging 17.5 mg. at 25° and 13.1 mg. at 20°C. The corresponding weights for freshly molted third instars were 79.7 mg. and 49.0 mg. In spite of this difference in weight, no difference in the number of columnar cells in larvae of the same stage of development was observed, however, in those reared at the higher temperature, the size of these cells was definitely larger. The average volume of each cell in the freshly molted second instar larva was 1584 cu. $\mu$ at 25° and 1172 cu. $\mu$ at 20°. For the freshly molted third instar larva, the corresponding figures were 2404 cu. $\mu$ and 1863 cu. $\mu$. These observations are opposed to the commonly accepted belief that the size of cells and of cell constituents is larger in organisms reared at lower temperatures.

### 191. A HORMONE AFFECTING VIABILITY IN CRUSTACEANS. Frank A. Brown, Jr., Northwestern University.

The crayfish, Cambarus blandingei acutus, was observed to survive, on the average, a significantly shorter time when the two eyestalks were removed than when in possession of one or both eyestalks. There appeared to be no significant difference in survival time between crayfishes from which a single stalk was removed and a completely normal one. A test was made for the assumption that the eyestalks normally liberate into the blood a substance essential for the well-being of the animal. Two lots of crayfishes were deprived of both eyestalks. At the time of this operation the contents of the animal's own eyestalks macerated in Ringer's solution were implanted into the ventral abdominal region of each animal in one lot. Into each animal of the other lot a similar quantity of Ringer's solution was injected into the ventral abdomen. The eyestalkless crayfishes which received the stalk tissue implants lived significantly longer than those receiving the Ringer's solution alone. This was taken to be sufficient evidence that a hormone essential to the continued life of the crayfishes was formed within the eyestalks.

### 192. AFTER-EFFECTS OF PITUITARY INJECTIONS UPON THE MALE GENITAL SYSTEM OF ANOLIS CAROLINENSIS. Llewellyn Thomas Evans, Montana State University.

Out of the twenty-nine males (weighing between 3 and 3.5 gm.) used, seven served as controls and twenty-two were divided into two groups of thirteen and nine, respectively. Group 1 received eight intraperitoneal injections (1 r.u. per injection) of whole sheep pituitary extract at 36-hour intervals. Four of these were sacrificed within 12 days (average weight of testes, 27.0 mg.) while nine were killed at intervals between 72 and 160 days after the last injection (average weight of testes, 0.9 mg.). Group 2 received eight injections of Antuitrin S (1 r.u. per injection). Eight of these were killed within a week (average weight of testes, 14.8 mg.) and one after 53 days after the last injection (weight of testes, 0.4 mg.).

The after-effects of injections revealed marked destruction and atrophy of testicular tissue. Spermatogonia were the last to be affected. In contrast the epididymis and vas deferens were uniformly larger in diameter than those of controls, approaching the degree of hypertrophy reached immediately after the injections were given.

### 193. THE EFFECT OF HYPOPHYSECTOMY ON THE TESTIS OF FUNDULUS. Samuel A. Matthews, Williams College.

The pituitary gland was removed from 176 Fundulus of which records were obtained from thirty-three males. One group was hypophysectomized in the fall, when the testis is normally small, and allowed to run until the next breeding season in May and June when it was found that the testes had failed to enlarge as did those of control animals. Microscopically these testes showed numerous primary spermatogonia, but very few spermatocytes and no spermia. A second group was operated on in March and April after the testes had already enlarged somewhat. After 14 to 21 days the testes became markedly smaller than those of control animals and also showed only primary spermatogonia with practically no spermatocytes but in these cases a few spermatids and spermia were present. The pituitary gland appears to play a role in controlling the seasonal cycle which the testis of this teleostean fish exhibits and is apparently of particular importance in the maturation of spermatozoa.

### 194. Some effects of hypophysectomy in chick embryogenesis. Nicholas W. Fugo, State University of Iowa. (Introduced by Emil Witschi.)

The primordia of the pituitary was removed by means of glass needles from embryos of 33 to 38 hours of incubation in an effort to determine the endocrine role of the embryonic hypophysis during development and more especially its importance in the embryogeny of the thyroid and the genital system. Of 429 operations 123 embryos lived beyond the twelfth day. Operated and control animals were preserved from the twelfth to twenty-first day of incubation.

The hypophysectomized animals have normal body proportions but are smaller than controls of the same age, the difference in weight becoming more apparent as incubation progresses. The feather papillae of the experimental embryos are much darker than those of normal controls. The thyroids first form normally but later remain deficient in colloid to varying degrees showing small undeveloped follicles. No gross abnormalities were observed on histological examination of the parathyroid or thymus glands.

The gonoducts develop normally in both sexes. Regression of the mullerian ducts in the male occurs at the normal time and in the usual manner. The right oviduct regresses normally in the female. The testes of the experimentals contain a smaller amount of stromal tissue than the controls, while no differences could be observed between female gonads of hypophysectomized and normal animals. Observations on adrenals indicate no differences between experimental and control embryos.

### 195. Effect of pituitary implants on the mammary glands of hypophysectomized rats. Warren O. Nelson, Department of Anatomy, Wayne University, College of Medicine.

Large groups of adult male and female rats were injected with estrogenic hormone (300 to 1000 I.U. daily) for 15 and 30 days. Their anterior pituitary glands were implanted into hypophysectomized young female rats. Implants were made daily for 21 days and at autopsy the mammary glands and ovaries were studied and compared with tissues from a parallel series of animals which had received implants of pituitaries from normal animals. Although the animals treated with tissue from estrin-injected rats received almost twice as much pituitary material (due to the great increase of pituitary weight with estrin treatment) as the control series, their mammary glands were, on the whole, less well developed and their ovaries were smaller than those of the control series.

In several animals of each series 100 I.U. of estrin was given in addition to the implants. Mammary glands were better developed in this group, but again there was no evidence that the pituitaries of the estrin-injected donors were richer in a 'mammogenic hormone' than the pituitaries of untreated animals. This study fails to confirm the claim for the existence of a specific 'mammogenic hormone' or that the production of such a hormone is stimulated by the injection of estrin.

### 196. Effect of progesterone on the testes of hypophysectomized rats. Warren O. Nelson, Department of Anatomy, Wayne University, College of Medicine.

The positive effect of androgenic hormone on the maintenance of spermatogenesis in the hypophysectomized rat and mouse has been demonstrated and confirmed. On the other hand, estrogenic hormone, even in the normal animals, suppresses testicular function.

To test the effect of corpus luteum hormone on the testes, twenty-three adult rats were hypophysectomized and injected for 20 days with progestin containing extracts or crystalline progesterone. It was observed that at least 2 mg. of progesterone or its equivalent of extract was required to show any significant effect on maintenance of spermatogenesis and testicular weight.

Two series of four animals each received 2 and 3 mg., respectively, of crystalline progesterone daily. Their testes at autopsy averaged 1.288 and 1.623 gm., respectively. Active spermatogenesis was in progress in each instance and motile sperm were present in the epididymidi. Testes of untreated hypophysectomized controls averaged 0.530 gm. and sperm were not found in testes or epididymidi. A slight, but regular, androgenic-like effect of the treatment on the sex accessories was observed.

### 197. The response of the gonads of nestling passerines (Passer domesticus and Agelaius phoeniceus) to gonadotropic substances. Gardner M. Riley, State University of Iowa. (Introduced by Emil Witschi.)

Starting on the day of hatching, sparrows and redwing blackbirds were injected intramuscularly with several gonadotropic preparations. Testis enlargement followed injections of pregnant mare serum, anterior pituitary suspensions (horse, bull and rat) and a follicle stimulating preparation extracted from sheep anterior pituitary. Both intertubular and tubular stimulation followed injections of pregnant mare serum. Injections of anterior pituitary preparations were followed by tubular stimulation primarily, with some intertubular hypertrophy. Injections of the follicle stimulating fraction of sheep pituitary resulted mainly in tubular development. Although the epididymes and vas deferentia of injected males were enlarged, the bills showed no color change. No definite effect of the various gonadotropic preparations on the ovaries could be noted. The results emphasize the fact that the bird testis is more responsive to gonadotropic substances at all times than the ovary.

### 198. Effect of theelin administration on female sparrows. A. R. Ringoen, University of Minnesota.

This particular study was designed in the light of earlier experimental results relative to the effect of theelin administration on the reproductive system of English sparrows during the months of January and February. Precocious oviduct hypertrophy was observed subsequent to the repeated injections of the estrogenic substance.

Oviduct response to hormonal activation has recently been followed in theelin treated sparrows during October. The trapped birds were brought into the laboratory and kept until October 12th, when the experimental work was begun.

Daily injections of theelin in oil in dosages of 0.1 cc. were made into the breast muscle alternating the site of injection from day to day into the right and left sides of the breast. Uninjected specimens served as controls. The theelin treated birds showed precocious development of the oviduct. Significant duct response was observed after the ninth injection of the hormonal substance. Juvenile as well as sexually quiescent female birds responded to the estrogenic substance. It is therefore concluded that during the fall time female sparrows are not refractory to injections of female sex hormone.

### 199. EFFECTS OF INJECTIONS OF EPINEPHRINE ON THE SOCIAL ORDER IN SMALL FLOCKS OF HENS. W. C. Allee and Nicholas Collias, The University of Chicago.

Hens exhibit a relatively stable social order. Rank is determined by active combats between individuals or by more or less passive submission. To explore the underlying physiological basis, an attempt was made to effect changes in the peck-order of White Leghorn pullets approximately 9 months old, which had been under observation for 86 days as members of four small flocks.

From 0.5 cc. of 1: 5000 to 1.5 cc. of 1: 1000 epinephrine was daily injected subcutaneously into a bird of mid-rank in each of three flocks for 10 to 26 days. Uninjected controls and birds injected with 0.8% NaCl solution were also observed. These injections produced no change in social rank or in relative number of pecks received or delivered. The treatment was working against conservative forces of habit which tend strongly to stabilize a peck-order once it is established.

Ten birds from two flocks were kept isolated for 28 days or longer and then introduced two together into a strange pen. In the case of birds from flock 1, and in inter-flock contacts, the individual lower in social rank or, if equal, the lighter bird, was injected with epinephrine from 0.25 to 3 hours before meeting its opponent for the day. Birds from flock 2 were non-injected controls for intra-flock contacts.

At the dosages used, former high social rank or greater weight, rather than injections of epinephrine appear to be associated with greater success in these initial meetings which determine the social order.

### 200. THYROIDECTOMY AND GONAD RESPONSE TO GONADOTROPIC HORMONES. Samuel L. Leonard, Rutgers University.

Thyroidectomy was performed on immature female guinea pigs weighing about 200 gm. After several days the operated and control animals received injections of follicle stimulating hormone (menopause urine extract) and of pregnancy urine extract. After 4 or 5 days of treatment the animals were killed and ovaries weighed. The amounts of hormone given increased ovarian weights by 50% or more. A typical experiment gave average ovarian weights of four thyroidectomized animals injected with F.S.H. equal to 169.2 mg. compared to 119.3 mg. for six controls. With pregnancy urine injections, the ovaries of five operated animals averaged 79.4 mg. as compared to 84.7 mg. for three controls. Thus, thyroidectomy in the guinea pig, as in the rat, enhances the action of F.S.H. but not the action of pregnancy urine extract.

Five young male chickens (5 weeks old) were thyroidectomized. After 3 days, a total dose of 1 gm. of alkaline extract of sheep hypophysis was injected into each bird and into three controls. Two other controls received no injections. Average testes weights after 5 days treatment were: 210 mg. for non-injected controls; 458 mg. for injected controls; 251 mg. for three completely thyroidectomized birds; and 388 mg. for two which were incompletely thyroidectomized. Because of uniformity of results, in spite of the few numbers, it is concluded that chickens differ from guinea pigs and rats in showing a decreased response to hypophyseal gonadotropic hormone after thyroidectomy.

### 201. Effects of androgens on fish (Lebistes reticulatus). Wilburn J. Eversole and Robert Gaunt, Washington Square College, New York University.

One hundred fish (Lebistes reticulatus) were treated with testosterone propionate for periods varying from birth to maturity. Fish were kept in 2½-gallon aquaria in groups of from four (if adults) to sixteen (young broods). Two-thirds of the fish were injected twice weekly, by means of a micro-injection apparatus, with approximately 0.03 mg. of testosterone propionate over a period ranging from 1 week to 4 months. One-third were hormone-fed by adding testosterone in ether solution to the food and then evaporating the mass to dryness. Feeding was done in such amounts that 0.5 mg. was added daily to each aquarium for periods varying from 2 to 3½ months.

Female fish fed or injected with the hormone readily assumed some but not all of the male secondary sex characters. Testosterone administration induced gonopod (male intromittant organ) formation in females at any stage of development but did not induce the typical male red, yellow and orange body pigment. Growth in the female was apparently inhibited (males are normally much smaller than females) but the gravid spot, a distinctive female trait, could not be suppressed.

As a result of hormone treatment the female gonad was markedly reduced in size showing inhibited gametogenic activity and a resorption of yolk and embryos if present. The effects observed in the male gonad were too variable for accurate interpretation until dosage and treatment duration factors are more fully studied.

### 202. Influence of sex hormones on sex differentiation in amphibia (Rana pipiens). Charles L. Foote, State University of Iowa. (Introduced by Emil Witschi.)

A study was made of the influence of sex hormones, placed in the aquarium water, on sex differentiation in Rana pipiens tadpoles.

One group, treated for 65 days from the time of hatching, received 24 I.U. of crystalline testosterone propionate (Perandren) daily; another group received 100 R.U. of estrone (Theelin) daily; and a third group received 8000 I.U. Cristallovar I.B.I. twice daily. Units of hormone as given are per liter of water; each group consists of twenty-four animals. Similar treatment with testosterone and estrone was begun on a second series 36 days old, and continued for 28 days.

Histological examination of the gonads showed that all tadpoles subjected to testosterone were males. Of those treated with estrone, 15.5% of the first series were females, 67% showed testes containing ovocytes, and 16.5% were undifferentiated; in the second series 45% were females and 55% showed testes with ovocytes. Of those treated with Cristallovar 75% were males, 5% showed testes with ovocytes, and 20% were undifferentiated. Control groups for the first series were 70% males and 30% females; for the second series 44% males and 56% females.

These results indicate that testosterone propionate and Cristallovar promote testis differentiation in females, while estrone stimulates ovocyte development in the testes of males.

### 203. HYPERTROPHY OF TURTLE THYROIDS INDUCED BY MAMMALIAN WHOLE PITUITARY EXTRACT. Llewellyn Thomas Evans, Montana State University.

Twenty-seven adult turtles (collected within 30 miles of Missoula, Montana) were used. Seven females and three males served as controls, while ten females and three males received from 4 to 20 daily subcutaneous injections (single injection, 0.025 gm. dry weight) and were sacrificed 24 hours after the last injection. Four females received an average of thirty-six injections daily for the first 30 days and then one injection every 4 or 5 days thereafter. These four were sacrificed 2½ months after the last injection.

Histological examination showed the following measurements which represent the average of at least 120 individually measured follicles and the same number of individual cell heights for each of the twenty-seven turtles. In control females the diameter of the follicles was 154.6 $\mu$ and the height was 9.3 $\mu$. In control males the follicles were 112.4 $\mu$ in diameter and 8.1 $\mu$ in height. In the group of ten injected females the diameter of the follicles averaged 102.4 $\mu$ and the cell height averaged 15.7 $\mu$. Injected males averaged 88.2 $\mu$ in follicular diameter and 17.0 $\mu$ in follicular cell height. The group of four injected females averaged a follicular diameter of 98.2 $\mu$ and a cell height of 18.2 $\mu$.

It is shown that pituitary stimulation decreases diameter, but increases the height of follicular cells. The after effects of injections accentuate hypertrophy.

### 204. STUDIES ON THE REPRODUCTIVE TRACT OF THE MALE BOX TURTLE. Ira B. Hansen, The George Washington University.

The seasonal variation in the male reproductive tract of Terrapene carolina follows closely that described by Risley for the Musk turtle. The testes are smallest in April and early May. Spermatogenesis begins and the testes gradually enlarge about the middle of May. Very few secondary spermatocytes appear until the last of June, and become abundant during July. Spermiogenesis begins toward the end of July when the testes have reached their maximum size and turgidity. Attached spermatozoa are characteristic of the testes through August when the testes become soft. The cycle of spermatogenesis completes itself toward the end of September and the walls of the seminiferous tubules are reduced to the basal layers of cells. Testicular regression in size follows through the winter months.

Castrations covering periods from 10 days through 3 months have been performed with decided reduction in the total volume of the epididymis. This is largely due to the decrease in the volume of sperm although residual spermatozoa are still alive and vigorous at the end of 74 days of complete castration. The height of the epithelium of the epididymis shows marked normal variations from about 5 to 32 $\mu$. Short time castrations are without effect but long time castrations tend to reduce the height of the epithelium to its lower limit. This result is not as yet decisive.

205. **Adrenal cortex and diabetes insipidus.** Malvina Schweizer, Robert Gaunt, Warren O. Nelson and Eleanor Loomis, Department of Biology, Washington Square College, New York University; Department of Anatomy, Wayne University; and Biological Laboratory, Cold Spring Harbor.

Silvette and Britton, studying normal and adrenalectomized opossums, advanced the hypothesis that the cortical hormone is a diuretic agent antagonized physiologically by the anti-diuretic secretion of the posterior lobe. They suggested that the inactivation of the adrenal cortex was the reason for the typically transitory nature of the diabetes insipidus which usually follows total hypophysectomy.

With somewhat similar possibilities in mind we had independently carried out experiments which tested the latter part of their hypothesis and failed to confirm it. Ten completely hypophysectomized rats were given 6 to 9 mg. per day (from the day of hypophysectomy for 7 to 14 days) of an adrenotropic-lactogenic hormone combination which maintained adrenal size above normal levels. Six of these cases showed no marked polyuria, and the treatment failed to prevent cessation of diabetes insipidus in the four cases where it initially appeared.

Four hypophysectomized animals in which diabetes insipidus had occurred and subsided were then given a purified adrenotropic preparation (4 mg. per day for 7 days), and in these cases this treatment reduced the urine output instead of reinstituting the polyuria. Three cubic centimeters per day of cortical extract in two similar cases likewise showed no tendency to return the polyuria.

It is concluded that in the rat at least the partial inactivation of the adrenal cortex following hypophysectomy is not the cause of the subsidence of the polyuria.

Pituitary hormones were prepared in the research laboratories of Parke, Davis & Co.

206. **Effect of bilateral optic enucleation on certain reproductive phenomena in rats under various light conditions.** Ludvig G. Browman, Montana State University.

Female rats which have both eyes removed at birth have delayed vaginal introitus regardless of the light conditions to which they are subjected. Normal female rats under normal colony light conditions have the opening of the vagina on an average 11.2 days earlier than enucleated litter mates; in continuous darkness normal rats open 9.4 days earlier than enucleated litter mates, and in continuous light 12.7 days earlier than enucleated litter mates. Normal females sacrificed at 100 days of age have uterine weights that are consistently heavier than those of enucleated litter mate sisters.

Male rats which have both eyes removed at birth have seminal vesicles definitely lighter than those of normal litter mates, regardless of light conditions of the environment. Testicular weight is proportionately the same per unit of body weight at 100 days of age; but at 40 days testicles of enucleated animals are lighter than normal, and have very few sperm heads as compared with normal litter mate testicles. The epithelial height of seminal vesicles of enucleated males is somewhat less than that of normals, as is the amount of secretion, although secretion granules are nearly as abundant in both categories.

On the basis of percentage weights it is apparent that seminal vesicles and uterine weights in enucleated animals are lower proportionately than would be expected of animals whose weights are otherwise below normal as a result of enucleation.

### 207. A SIMPLE METHOD FOR THE SHARP DIFFERENTIATION OF THE BASOPHILIC GRANULES IN THE ANTERIOR PITUITARY. Sam R. Hall and John D. Hunt, Bureau of Dairy Industry, U. S. Department of Agriculture.

After more than a score of staining and fixing combinations were tried, Zenker formol or Zenker acetic, followed by what might be considered a modification of Mallory's phosphomolybdic acid haematoxylin with the two solutions kept separate, was found to stain sharply the basophilic granules in cattle. Although this basophilic stain may be used alone, it is best employed after orange G, acid fuchsin, or azo carmine in the Mallory azan method, or instead of aniline blue in the Masson technique.

More exactly, slides remain in a saturated solution of orange G in 2% phosphomolybdic acid for 15 or more minutes, then in 2% phosphomolybdic acid for 1 to 2 minutes followed by an aqueous solution of well-ripened haematoxylin for ½ to 2 hours. It is often well to add 1 to 2 drops of 10% acetic acid to the haematoxylin. Preparations now 2 years old have not faded.

That the haematoxylin in combination with phosphomolybdic or phosphotungstic acid is a basophilic and not an acidophilic or mitochondrial stain as it is in the iron or copper haematoxylin methods is demonstrated by examination of serial sections in combination with aniline blue, copper haematoxylin, etc.

The mitochondrial and Golgi techniques of Severinghaus have given splendid results.

### 208. POSSIBLE EVIDENCE FOR A SECOND TYPE OF BASOPHILE IN THE ANTERIOR PITUITARY OF CATTLE. Sam R. Hall, Bureau of Dairy Industry, U. S. Department of Agriculture.

The cells that might be called aniline-blue-strong or dark basophiles can be separated in cattle from the weak or light by the much greater affinity of the granules in the former for haematoxylin following phosphomolybdic acid. A cytological study of this 'haematoxylinophilic' cell in more than 170 cattle in several physiological conditions reveals other differences between it and the ordinary typical basophile.

The haematoxylin form is distributed over the entire gland while the cell typical in most respects of the large rat basophile is confined almost entirely to the central core. This scattered small cell almost always has a characteristically compact nucleus. Except in rare instances, few mitochondria are present and the Golgi apparatus even in the hypertrophied condition is not large.

Both basophiles appear at about 3 months' fetal life, after the acidophiles, and at this time may be distinguished by their granulation. Among the embryonic chromophobes the nuclear differences are also apparent. Golgi distinctions are not striking.

Up to 2 or 3 months after birth, the haematoxylin cells are fairly inactive, remaining filled with granules. Thereafter, with the onset of puberty in the normal animal, they become obviously more active and continue so throughout adult life.

This cell is apparently not present in the rat pituitary.

The typical basophiles have not been observed to undergo comparable morphological changes in normal animals. They are not static, however, since they enlarge and entensively vacuolate in the thyroidectomized-gonadectomized animal.

### 209. EFFECT OF CASTRATION ON THE BASOPHILES OF THE BOVINE ANTERIOR PITUITARY, WITH A CONSIDERATION OF THE FREEMARTIN. Sam R. Hall, Bureau of Dairy Industry, U. S. Department of Agriculture.

Contrary to the preponderance of evidence on the effect of castration in other animals, the present study has shown that the operation in cattle causes no increase either in number or apparent activity of the basophilic cells.

The size of the central basophilic core is not increased and the cells of this area, typical of the basophiles of most other animals, are not structurally affected by the operation. However, the small haematoxylin-granular basophiles invariably show granule accumulation, small negative Golgi image and a size decrease. This holds following either prepuberal or postpuberal castration. These scattered basophiles of the castrate anterior pituitary resemble those of the immature animal but are on the whole even less active, as judged by the usual standards.

The lack of activity in the 'haematoxylinophilic' cells in both the immature and the castrate animal, with the long time castrate being 'completely dormant,' might lead one to suspect that this cell is dependent upon the gonadal hormone(s) for its activation, and not for its suppression as is the case with the rat's basophile.

With this new criterion for castration effect in mind, seven freemartin anterior pituitaries were examined. None were typical of castration though three were nearly so. None, however, were typical of the normal cow.

### 210. THE MORPHOGENESIS AND CYTOGENESIS OF THE PARS BUCCALIS IN THE DUCK PITUITARY. Ben T. Painter, Harvard University. (Introduced by A. B. Dawson.)

The morphological and cytological changes in the pars anterior and the relation of the pars tuberalis to the infundibulum have been observed in the duck from 9 days of incubation to the adult condition. Externally, the pars anterior shows little evidence of a bilobed structure such as that exhibited by the chicken. A cytological study, however, shows certain cell types restricted to rather definite 'cephalic' and 'caudal' regions. The residual lumen disappears in the late embryonic stages. No pars intermedia has been found. The methods of fixation and staining were those used by Dawson. Clearly defined basophils, located in the cephalic portion of the pars anterior, are recognized about the sixteenth day of incubation. In later stages they appear in the caudal portion, but in most cases are more numerous in the cephalic. Acidophils appear in the cephalic region about the fifteenth day of incubation. Shortly after hatching, they become fairly evenly distributed throughout the gland. Later a 'shift' seems to occur; they become more numerous in the caudal region.

### 211. CYTOLOGICAL ABNORMALITIES IN THE TESTES OF PITUITARY-COLCHICINE INJECTED RATS. Opal Wolf, Goucher College.

Mitotic activity of the endocrine glands of the rat has been studied following injections of an alkaline extract of the anterior lobe of the pituitary[1] at 1 cc. and 2 cc. levels. Subcutaneous injections of 0.3 mg. of colchicine[2] dissolved in distilled water were given 9 to 15 hours before autopsy. The animals were killed on the second, fourth, sixth and tenth days of the experiment, the tissues were fixed in Bouin's and stained in Harris' and Heidenhain's haematoxylin. In previous reports on the adrenal glands cells in metaphase and various stages leading to multinuclear cells were reported. Displaced spindles were also shown. Various cytological abnormalities have been noted in the testes such as tripolar and multipolar spindles. Many cells showed an uneven distribution of chromatin on the single spindle and in many cases chromosomes were noted in the center of the spindle at late telophase or free in the cytoplasm. As a result of these abormalities bi-nucleate cells with two small or a large and small nuclei were seen, also tri-nucleate and tetra-nucleate cells. In some cases the tubules of rats injected 10 days showed many normal spermatids with occasional very large cells near the lumen. Eight centrioles could be counted in these cells. It seems evident that these large spermatids must contain the tetraploid number of chromosomes. A division of the chromosomes appears to have taken place together with the corresponding divisions of the centrioles resulting, however, in the formation of one large nucleus with no division of the cytoplasm.

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[1] The growth extract was purchased from E. R. Squibb and Sons.

[2] Colchicine U.S.P. was purchased from Merck and Co. in the form of a powder.

### 212. EFFECTS OF LOW TEMPERATURES ON SALIVARY GLAND CHROMOSOMES. John B. Buck and A. M. Melland, Carnegie Institution of Washington, Baltimore.

From experiments on the preparation of undenatured salivary gland chromosomes of Chironomus the following tentative conclusions can be drawn:

1. Almost invariably in fresh nuclei which have been rapidly frozen at —70°C. and thawed, the chromosomes are disrupted and the entire nucleus is filled with narrow parallel ridges, furrows or 'striations,' presumably due to the formation in the nuclear contents of long needle-like ice crystals in parallel orientation. Striations may occur at any temperature between —70°C. and —30°C., although less frequently at the latter.
2. The striations are not associated with the thawing process, as they appear in material dehydrated at —30°C. after freezing at —70°C.; nor are they associated with either the dissection medium or the freezing agent, but only with the low temperatures.
3. The disruption of chromosome structure and the formation of striations decrease as the amount of water in the chromosomes decreases.
4. Although freezing disrupts chromosome structure it apparently does not denature the nuclear proteins as they still react to solutions of various osmotic concentrations by shrinking or swelling reversibly. Curiously, in order to be isotonic with the chromosomes, a solution need be only about half as concentrated after the gland is frozen as before. This is due presumably to the disruption of the cytoplasm which acts normally as an osmotic insulator to the nucleus. Freezing thus produces a physical rather than a chemical change.
5. Chromosomes denatured previous to freezing are relatively unaffected by low temperatures.

### 213. STUDIES OF IMPRINT PREPARATIONS OF PIGEON BONE MARROW. A. R. Ringeon, University of Minnesota.

Imprint preparations are being rather widely used at the present day in cytological studies of human bone marrow (sternal biopsies). The chief advantage of the imprint method is that it brings out the finer structural features of the nucleus which are so important in the morphological identification and interpretation of immature cells. The difficulties encountered in studies of sections of pigeon bone marrow lead to the adoption of the imprint method.

The cytological evidence obtained from studies of the marrow imprints substantiates the deduction that pigeon marrow is definitely erythroblastic (normoblastic) and not megaloblastic as claimed by a number of investigators. Regeneration of the red cells is largely by homoplastic means although heteroplastic development occurs as evidenced by the transitional stages between the stem cell and red cell series.

The morphological features of the stem cell are similar to those of the mammalian parent cell. The proerythroblast exhibits a decidedly basophilic cytoplasm; the nucleoli are single or multiple. The characteristic features of the basophil erythroblast are pronounced basophilia; absence of nucleoli; nuclear structure erythroblastic. Polychromatophilic and orthochromatic erythroblasts are readily identified.

Summarizing: the 'lymphoid' cells of pigeon bone marrow represent stem cells and early stages (proerythroblasts and basophilic erythroblasts) in red cell development. Although the stem cell is retained as a potential source of the red cell series, the more common type of erythrocyte production is by homoplastic development.

### 214. A STUDY OF FIBRIL FORMATION IN CONNECTIVE TISSUE, BY THE METHOD OF DISSOCIATION WITH POTASSIUM HYDROXIDE. H. E. Jordan, University of Virginia.

The umbilical cord of 10- to 15-mm. pig embryos is the material of this study. Stained sections show areas of varying density, with varying amounts of intercellular fibrils. Both intracellular and extracellular collections of fibrils include coarser and finer varieties. Many of the coarser fibrils become resolved terminally into a brush of finer fibrils. Many of the 'extracellular' fibrils are continuous with cell processes. Sections alone are inadequate for solution of the problem of fibrillogenesis: whether intracellular or intercellular. Dissociation of the tissue with caustic potash solutions and subsequent staining with brilliant cresyl blue give clear evidence of the intracellular location of the initial fibrils; no free (intercellular) fibrils occur in the preserving fluid (glycerine-alcohol). Many of the cells have enormously long processes, consisting almost entirely of fibrils. Certain processes are essentially a coarse fibril or a compact bundle of fibrils which break into a brush of delicate fibrils terminally. Initial coarser fibrils arise in the exoplasm of the fibroblasts and split into secondary finer fibrils. These fibrils are argyrophil as revealed in sections stained with Foot's reticular connective tissue technique, and cannot be regarded as 'tonofibrils' of Maximow or 'fibroglia' of Mallory.

### 215. ANGLE OF CAPILLARY BRANCHING AND ENDOTHELIAL VALVES. Benjamin W. Zweifach, New York University. (Introduced by Robert Chambers.)

The architecture of the capillary system is such that the arrangement of two types of vessels is a primary factor for the local distribution of capillary blood. The arteriolar channel not only gives off capillary side branches, but continues through without interruption (a–v bridge) to the venule. The true capillaries come off at successively different angles. Those on the arterial end come off abruptly and curve sharply backward. The peculiar angle of branching suddenly and markedly slows the flow of blood into the true capillaries. Further distally, the less and less acute angles at which the successive capillary branches leave the vessel is paralleled by a gradual diminution in the rate of flow through the a–v capillary.

A valve-like fold of endothelium is present at the point of capillary exit in those regions where the capillary offshoots have a sharp backward angle. Movements of the a–v wall occur during periods of extended increase or decrease in blood flow through the vessel, and these alter the degree to which the endothelial folds obstruct the orifice into the capillary offshoot.

This structural set-up allows during ischemia a continuous flow only through the central a–v channel, the flow through the true capillaries being sporadic. During hyperemia, dilatation of the a–v bridge opens the endothelial valves of the true capillaries and introduces a flow of blood throughout the entire capillary system.

### 216. REGENERATION IN THE CENTRAL NERVOUS SYSTEM OF FUNDULUS HETEROCLITUS EMBRYOS AFTER CAUTERY. S. M. Nabrit, Morehouse College. (Introduced by J. W. Wilson.)

Four- and 5-day-old Fundulus heteroclitus embryos were cauterized in the brain region by a glowing hot needle. Chorions were left intact. The embryos were killed daily following cautery up to hatching time. Specimens of each age group were examined histologically.

Cautery produced injuries that varied with the temperature of the needle and the time of exposure. As much as 50% of the brain could be destroyed and development would continue.

Cautery not only killed many cells of the neural epithelium but separated many cells. The separated cells showed vigorous mitotic activity and produced many cells that aided in the replacement of injured area. These were probably the first cells to participate in the active repair.

In severe cautery, numerous cells in cord and hind brain assumed the early prophase states common in neural tube mitosis. The prophases under these conditions were not limited to the vicinity of the lumen as much as is the case in the normally developing tube. Several histological observations indicated that the activated cells wandered to the region of injury: 1) the large number of cells active in regions removed from the primary injury; 2) the presence of them in the lumen; and 3) the progressive increase in them near the zone of injury as the age of embryos increased.

As regeneration neared completion, the heightened mitotic activity was limited to that portion of the neural tube requiring additional material for completion. There were accompanying color and structural differences in the old normal, activated and new tissues to make them easily distinguishable from one another.

Frequently ruptured capillaries caused the cavity of the brain to become infiltrated with red blood corpuscles. Neural epithelium adjacent to some of these blood patches showed heightened mitotic activity, but this likewise was in regions in process of repair.

It appeared that many of the malformations noted in the regenerates were due to head malformations brought about by the injuries rather than lack of capacity for the neural epithelium to reform itself after cautery.

### 217. SOME OBSERVATIONS ON THE HISTOLOGY OF THE DIGESTIVE TRACT OF THE CRAWFISH, CAMBARUS VIRILIS HAGEN. Arno F. Michaelis, Marquette University. (Introduced by Paul L. Carroll.)

The epithelium of the esophagus consists of simple columnar cells which lie beneath a thin layer of chitin. This chitinous material lines the entire lumen and consists of two layers, the first is narrow but very dense, while the second is somewhat irregular in contour and less dense. The columnar cells vary in size from tall to low columnar. The nucleus is usually round, although at times rectangular in shape. The nucleus is located in the lower half of the cell, but with a tendency to approach the center. The chromatin is distributed rather evenly in the body of the nucleus, and one or more nucleoli are present. At the base of the columnar cells there is a layer of dense reticular connective tissue through which blood vessels, sinuses and nerves course. Compound saccular glands are found in this layer of connective tissue. There are two layers of muscles, the longitudinal multinucleated striated fiber bundles surrounded by masses of connective tissue, and the circular less dense fibers resting beneath the serosa-like pavement epithelium coating.

The cytoplasm of the columnar epithelium of the stomach is vacuolated and at times granulated; the nuclei are located at the base of the cell; a basement membrane is usually present. There are four distinct lamellae in the teeth of the gastric mill.

### 218. THE NITROGEN REQUIREMENTS OF LOBOMONAS PIRIFORMIS. Kenneth L. Osterud, New York University. (Introduced by R. P. Hall.)

Several attempts have been made to grow Lobomonas piriformis (Pringsheim strain) in inorganic media. A bacteria-free stock culture in peptone medium furnished the initial inocula. The method of serial transfer was followed in order to dilute gradually the organic materials carried over from the original stock. The most satisfactory medium, containing $NH_4NO_3$ as a nitrogen source, has supported growth through ten successive transfers. At the tenth transfer the calculated peptone concentration had been reduced to approximately $10^{-18}$ gm. per cubic centimeter. Initial counts were made for each transfer, and final counts were made after each incubation period of 2 weeks. The ratio of final to initial counts ($X/X_o$) was used as an index of the growth rate. The growth rate showed no decrease in the later transfers, and it is evident that Lobomonas piriformis is capable of autotrophic nutrition.

In additional experiments, the inorganic nitrogen source has been replaced by either glycine or asparagin. Although the results are not yet conclusive, an increased growth rate indicates that either glycine or asparagin may serve as the sole source of nitrogen.

### 219. GROWTH OF CHLOROGONIUM TETRAGONIUM AT DIFFERENT HYDROGEN-ION CONCENTRATIONS. John B. Loefer, Berea College.

A medium consisting of 1% Bacto-tryptone and inorganic salts was prepared and portions of it were adjusted to hydrogen-ion concentrations ranging from pH 4.2 to 8.8. Equal amounts were pipetted into Pyrex culture tubes and autoclaved. All were given a 1 milliliter inoculum from a suspension of a bacteria-free stock culture grown in a Bacto-tryptone medium at pH 6.7. The cultures were incubated at 28°C. for 70 hours in constant light, after which the average number of organisms for each set of conditions was determined by use of a Whipple micrometer and a Sedgwick-Rafter counting chamber. The greatest number of organisms was observed at pH 8.6, average of the cultures being 193 per $mm.^3$ as compared with 155 at pH 8.3, 130 at 7.8, 121 at 7.2, 108 at 6.7 and 10 at 5.1. No increase occurred at pH 4.4. The optimum pH for this species is, therefore, considerably higher than that for C. euchlorum and C. elongatum. Both of these organisms grew best near pH 7.5 in the same type of medium.

### 220. EFFECT OF SOME 'EXCRETORY' COMPOUNDS ON GROWTH OF CHILOMONAS, CHLOROGONIUM AND GLAUCOMA. John B. Loefer, Berea College.

The following substances were added to a synthetic inorganic salt solution containing 1% Bacto-tryptone: allantoin, alloxan, benzoic acid, creatinine, hippuric acid, uracil, urea and uric acid. Portions of the basic medium at pH 6.7 containing 0.01 M concentrations of these substances were tubed, and sterilized in the usual manner. Six to twelve tubes containing each substance were then inoculated from a bacteria-free stock culture with sterile pipettes. Cultures of G. piriformis and C. paramecium were incubated 45 and 68 hours, when the average number of organisms was determined (with a Whipple micrometer and Sedgwick-Rafter counting chamber) for each group of cultures. With none of the above substances was growth better than in the controls when 0.01 M concentrations were used, and with benzoic, uric and hippuric acids toxic effects were evident. When more dilute concentrations, ranging from $1 \times 10^{-9}$ to $1 \times 10^{-3}$ M, were tested, no effect was produced on the growth of C. euchlorum or G. piriformis with allantoin. Uric acid, however, appeared to support slightly better growth just below the toxic concentration. The remaining compounds are being tested over a similar range of concentrations.

### 221. THE SELECTIVE ACTION OF INORGANIC MEDIA IN BACTERIA-FREE CULTURES OF EUGLENA. R. P. Hall and H. W. Schoenborn, New York University.

In the establishment of autotrophic strains from pure lines of Euglena anabaena var. minor, E. gracilis and E. viridis previously carried in peptone media, an apparent selection has occurred. In the first few transfers in inorganic media both the growth rate and the total population decreased sharply. Microscopic examination showed that the majority of flagellates lacked chlorophyll and were otherwise abnormal in appearance, while a few remained bright green and active.

In the third or fourth transfer there was a marked increase in growth rate and in total population, and the flagellates were almost exclusively the green, healthy type. This type has been predominant in later transfers. The observations suggest that, upon transfer of strains of Euglena from peptone media to inorganic solutions, there is a selection of a comparatively small proportion of faculatative autotrophs, while the rest of the population dies in the first few transfers.

### 222. Coccidian infections of western painted turtles of the Okoboji region.

Orville J. Deeds and Theodore Louis Jahn, State University of Iowa.

In a survey of 150 western painted turtles (Chrysemys marginata belli) 56% were found to be infected with one or more species of coccidia. Species found were:

Eimeria chrysemydis n. sp. Incidence 34%. Oocysts oval, $23\,\mu \times 15\,\mu$; differs from E. delagei in that the granular mass does not form a crescent and vacuole, in that there is no definite arrangement of sporocysts, and in host and geographical distribution.

Eimeria delagei var. marginata n. var. Incidence 6.7%. Differs from E. delagei in that segmentation does not result in five equal spheres and in that there are differences in size, shape, host and geographical distribution.

Eimeria mitrarium (Laveran and Mesnil). Incidence 32%. This is a new host and geographical record for the species.

### 223. The role of temperature, drying, and lethal factors in the hatchability of sexual eggs of Daphnia longispina and of Moina macrocopa.

Thelma R. Wood, Brown University.

Experiments to hatch 16,448 sexual eggs obtained in the laboratory from thirty-five clones of Daphnia longispina and Moina macrocopa have yielded evidence as follows.

Supposedly advantageous exposure of D. longispina sexual eggs to low temperatures did not alter their hatchability. Prolonged exposure was deleterious.

Hatching without previous drying occurred abundantly among D. longispina and Moina macrocopa sexual eggs. Prolonged storage dry, 5 months or longer, was decidedly harmful to D. longispina sexual eggs.

D. longispina sexual eggs stored wet retained their viability 7 years.

The presence in D. longispina clones of lethal factors reducing hatchability of their eggs is clearly indicated by 1) lower hatchability of eggs obtained by inbreeding, contrasted with higher hatchability of eggs obtained by crossing the same clones; 2) relatively high hatchability of eggs obtained by inbreeding clones early in their parthenogenetic history, contrasted with lower hatchability of eggs obtained by inbreeding the same clones after longer periods of parthenogenesis; and 3) death before hatching of many of the developing eggs obtained by inbreeding one clone, the number dying agreeing closely with that expected if one lethal factor, effective after resumption of development, were present in that clone.

The largest percentages of hatches were secured in groups which were kept wet, had the culture medium changed at intervals, and were aerated frequently in the course of microscopic examinations. Osmotic pressure and oxygen content of the medium appear to be the factors altered and hence would seem to play an important role in this procedure.

### 224. DEVELOPMENT OF EGGS OF THE JAPANESE BEETLE (POPILLIA JAPONICA NEWMAN) NEAR THE THRESHOLD TEMPERATURE. Henry Fox, University College, New York University. (Introduced by D. Ludwig.)

The published results of Ludwig indicate a temperature between 10° and 13°C. as the probable threshold of development for the egg of this species. Eggs incubated at 12° ± 1°C. showed clear traces of embryonic development in individual cases, but hatching occurred only when transferred to a higher temperature (27°C.) at 33 to 34 days after the beginning of incubation. Those retained at 12° for 54 to 75 days failed to hatch when exposed to the higher temperature. In cases yielding positive results, comparison with controls pointed to 0.00306 as the average daily rate of development during the period of exposure to 12°.

A limited number of eggs, incubated at high temperatures (24° to 27°C.) from 6 to 9 days immediately following deposition and then transferred to a temperature which gradually fell in the course of 5 weeks from 13° to 10°, hatched at intervals until the temperature reached 11.5°. Hatching, however, was often incomplete, the chorion splitting, but the larva failing to emerge or only partially freeing itself. Even in the few cases where hatching was apparently normal, the larvae were inactive and soon died.

These results show that eggs present, or larvae hatching, after the temperature of the ground in the autumn has fallen to 13°C. would not survive until the advent of favorable conditions in the following spring.

### 225. NUMBER OF EGGS IN OOTHECAE OF TWO INTRODUCED SPECIES OF ASIATIC MANTIDS AND THE PROPORTION HATCHING. Henry Fox, University College, New York University. (Introduced by D. Ludwig.)

Fifty-five oothecae of Tenodera sinensis Saussure collected in December, 1937, in the Bronx section of New York City gave egg counts ranging from 0 to 365, with an average of 261. Only one ootheca was empty and, as it was structurally abnormal, it seemed desirable to discard it, in which event the minimum count in this lot was 126 and the average 266. In a second lot of fifty-three oothecae from the Bronx, collected in March, 1938, the number of eggs ranged from 166 to 379, with an average of 265.

Twenty-seven oothecae of T. sinensis collected in December, 1937, at Ocean View, New Jersey, gave egg counts ranging from 230 to 351, with an average of 286, while seventy-one oothecae of T. angustipennis Saussure, collected with the preceding at the same place, gave egg counts ranging from 93 to 335, with an average of 234.

Discarding a few clearly abnormal cases, the proportion of eggs hatching in the three lots of T. sinensis was as follows: early Bronx, 92.2%; late Bronx, 87.5%; Ocean View, 94.5%. The wide range of variation in individual oothecae (49 to 100%) indicates these differences as of no statistical significance. In T. angustipennis the proportion hatching was 75.7%. From the standpoint of egg production the biotic potential in T. sinensis is indicated as higher than in T. angustipennis and in both as much higher than in the native mantid, Stagmomantis carolina (Johannson).

### 226. PODAGRION MANTIS ASHMEAD, AN EGG PARASITE OF INTRODUCED ASIATIC MANTIDS. Henry Fox, University College, New York University. (Introduced by D. Ludwig.)

In a lot of twenty-seven egg capsules of Tenodera sinensis Saussure and seventy-one of T. angustipennis Saussure from Ocean View, New Jersey, Podagrion mantis was present in one of the former and three of the latter. Only one dead parasite was found in the capsule of T. sinensis, occupying a cell near the attachment of the capsule to a twig. In those of T. angustipennis a total of sixty-one parasites was obtained, of which twenty-five were found in a capsule dissected before any of the parasites had emerged. Of the remaining thirty-six parasites, fourteen emerged as living adults, the others being found dead on subsequent dissection of the egg capsules. No parasites were found in 108 egg capsules of T. sinensis from the Bronx section of New York City.

Podagrion mantis has been long known as an egg parasite of the native Stagmomantis carolina (Johannson) and probably first spread to T. angustipennis where the range of the two species overlap, their egg capsules being similar in structure, with a thin outer covering easily penetrable by the ovipositor of the parasite. The relative freedom of T. sinensis from parasitism at Ocean View, where Podagrion occurs, is probably due to the thick, spongy outer covering of the egg capsule which makes oviposition by the parasite difficult except where the inner capsule enclosing the eggs lies near the surface, as along the line of attachment of the capsule to a twig or other support.

### 227. FOOD SPECIFICITY OF GRASSHOPPERS. F. B. Isely, Trinity University.

An analysis of the feeding behavior of forty common north-central Texas acridian species experimentally tested (1935–1938), shows that exactly twenty of the forty species were primarily grass feeders. The other twenty species chose their host-plants from among the forbs or broad-leaved flowering plants.

Many species of grasshoppers fed in cages died, if their specific host-plants were absent from the menu supplied; even when an abundance of fresh, green vegetation, acceptable to other acridian species, was provided.

Grasshopper food specificity, however, is not only a physiological fact but also a morphological adaptation. Microscopic and macroscopic examination of the mandibles of fifty species indicate that structurally they are exceeding variable, however, two chief patterns were found among the Acrididae.

In the grass eaters the anterior mesial margins of the mandibles are shallowly notched, the inner chewing faces are furrowed and ridged. Among the forbs feeders the mesial margins of the mandibles are sharply toothed, the mesial grinding areas are deeply denticular.

Among the longhorned grasshoppers (Tettigoniidae) the katydids, which were experimentally studied, fed chiefly on the flower part of plants. These katydids have relatively light jaws, and their mandibles have only small mesial grinding areas. The shield-bearers (Decticinae) studied were found to be chiefly carnivorous. These grasshopper-tigers have large heavy cycle-like mandibles.

### 228. OBSERVATIONS UPON CONCEALING AND WARNING COLORATION IN THE TROPICAL RAIN FOREST. Frank M. Hull, University of Mississippi.

The animals, particularly the insects and amphibians were observed along the trails of Barro Colorado Island in the Panama Canal Zone and were placed in classed according to the type of coloration they possessed or the type of behavior evidenced. A close relation between offensive and defensive types and conspicuousness was observed. Many moths in flying in the undergrowth were noted to invariably seek a green leaf for shelter. Others invariably chose dead leaves upon the trail. Many species habitually alight upon a leaf, only to quickly move to the edge and crawl under. Brilliantly colored wasp-moths (Syntomidae) when placed in cages with unfed ground lizards were carefully looked over and declined. Green katydids dressed with blue and red paint and placed with basilisk lizards were sometimes eaten and sometimes declined.

### 229. ON THE LIFE CYCLE OF A TAPEWORM, DIPHYLLOBOTHRIUM SP., FROM THE HERRING GULL, LARUS ARGENTATUS PONT. Lyell J. Thomas, University of Illinois.

Proglottids of a tapeworm, Diphyllobothrium sp., collected from time to time since 1930 along the shores of inland lakes in the United States and Canada, have been identified as belonging to a tapeworm from the herring gull. Complete adult worms which measure up to 49 cm. in length were collected from the young birds. Experiments show that the coracidia develop from eggs within 6 to 14 days depending upon temperature and other factors. Procercoids developed to the infective stage in Diaptomus sp. within 17 days. Cysts of plerocercoids from lake herring, Leucichthys artedi Le Sueur, fed to young gulls and a 6-week-old kitten were infective. Retention of the adult worm by the birds lasts less than 1 month.

### 230. THE LIFE CYCLE OF THE TYROGLYPHID MITES INFESTING CULTURES OF DROSOPHILA MELANOGASTER. Stanley G. Stolpe, State University of Iowa. (Introduced by Paul L. Risley.)

Tyroglyphid mites occurring in Drosophila cultures and on the flies themselves were identified as Histiostoma genetica sp. nov. The specific name, genetica, is suggested because of the prevalence of the mite in genetics laboratories.

Life history: the egg hatches within 24 hours into a hexapod larva, which, after a day, becomes an eight-legged nymph. There are two possible paths of development of the nymph: it may either develop into an adult directly in 2 to 9 days (the average being 6 days); or it may first metamorphose into an hypopus in from 1 to 4 days, which in turn becomes an adult after 1 to 9 days. The reason for the development along one path or the other is not known for both types are found together in the same culture. Adults copulate and eggs are laid in 2 to 7 days. However, isolated individuals have been observed from hatching, and these have laid eggs without the presence of males; hence parthenogenetic development is probable. The time required for a complete life cycle is from 6 to 16 days, depending upon conditions of moisture, temperature and food.

About 9 days are required under average conditions. The hypopus, that is found on flies, is a migratory form and is morphologically different from any other stage in the life history. It has a thick body covering, is resistant to somewhat adverse conditions, and derives no nourishment from its host because it has no mouth. However, it does attach to the Drosophila in such large numbers that the flies cannot feed or move, and hence death results.

### 231. RELATIVE GROWTH OF THE LIMB BONES IN THE ANTHROPOID APES. Hyman Lumer, Western Reserve University and Fenn College.

The relative growth of the limb bones in the gibbons, siamang, orang-utan, chimpanzee, and gorilla conforms to the equation $y = bx^{\alpha}$. Values of $b$ and $\alpha$ were obtained for the radius length/humerus length, tibia length/femur length, and leg length/arm length relations. In the gibbons, the constants for these relations are essentially the same in all the species investigated. The siamang resembles the gibbons in the first two, but differs from them in the third. These results indicate that in the evolution of the Hylobatidae, specific differences in adult proportions have arisen chiefly through mutations affecting adult body size but not the relative growth of the limb bones.

The orang-utan, chimpanzee, and gorilla differ for the most part in $b$ and $\alpha$. Sexual differences in adult proportions, however, are in general due to dissimilarity in adult body size in males and females, and not to differences in relative growth.

The values of $\alpha$ are close to unity except in the leg/arm relation in the gibbons and siamang, where $\alpha$ is considerably less than one. For the radius/humerus and leg/arm relations, $b$ is a decreasing exponential function of $\alpha$. The constants for the tibia/femur relation do not conform to this function, although there is an inverse relationship between them.

### 232. ARTERIAE CORONARIAE (CORDIS) IN THE HIGHER PRIMATES. Charles F. De Garis and Ralph E. Chase, Department of Anatomy, University of Oklahoma School of Medicine.

Among thirty-six hearts of higher primates studied as to the aa. coronariae, the following specimens have been examined: gorilla, two; chimpanzee, twenty-one; orang-utan, ten; gibbon, three. Coronary patterns in gorilla are fairly similar to each other and to that prevailing in man. In chimpanzee there is wide range of variation, involving especially the a. coronaria dextra; from one to three supernumerary branches from the right sinus aortae send distributions to the conus arteriosus or to conus and atrium dextrum; main courses of the coronary channels are frequently submuscular. In orang utan the variations are similar to those in chimpanzee, though supernumerary branches and submuscular courses are less frequent and extensive. In gibbon there is much diversity in distribution of the a. coronaria dextra, but supernumerary branches are not found in the three specimens examined; also the main channels are superficial.

Taking the series of higher primates as a whole, the right-sided coronary patterns present by far the greater variation. Topographical regions of the heart showing marked instability of arterial pattern are the conus arteriosus, atrium dextrum, sulcus atrio-ventricularis on the right side and sulcus longitudinalis posterior. The sulcus atrio-ventricularis on the left side, the sulcus longitudinalis anterior and sulcus coronarius are by comparison notably stable as to coronary patterns.

### 233. THE GROWTH OF THE PARTS OF THE UROGENITAL SYSTEM OF THE CAT. Homer B. Latimer, University of Kansas.

The kidneys, bladder, testes or ovaries and uterus were removed from 229 fetal, 35 newborn and 104 adult cats.

The growth of the testes forms a straight line curve when plotted on body weight (except a short initial period) and on body length. The ovaries plotted on body weight form a straight line curve. All of the other curves are concave superiorly.

Formulae have been developed for the ponderal growth in the fetal period of each organ based on body weight and body length. Only one formula has an average percentage deviation greater than 10%, or 11.5%.

The percentage weight of the kidneys increases rapidly at first and then more slowly with the adult average slightly less than in the newborn. The testes and ovaries are relatively heaviest in the early fetus, decreasing rapidly and then more slowly to birth. The adult ovaries are relatively a little lighter and the adult testes, relatively heavier than in the newborn. The uterus increases in relative weight to birth and is relatively lighter in the adult.

Only the kidneys and bladder are statistically heavier in the adult male cat. The adult kidneys have a higher coefficient of variation in the males and the bladder in the females. The adult kidneys have the highest correlation with body weight and the uterus and female bladder are the lowest. The average of the correlations with body length are lower, with the ovaries and uterus the lowest. These last two are positive but not significant.

### 234. CROSS SECTION ANATOMY OF THE HIND LIMB OF THE FROG. Wilfred F. Horner, Marquette University. (Introduced by Paul L. Carroll.)

There does not seem to be a complete study of the anatomy of the adult frog by means of cross sections, hence a series of sections through the limbs and the trunk of the common bullfrog have been prepared.

This section through the middle of the thigh is typical of all the sections made through the appendages. Cross sections of two regions of the trunk have been completed.

The diameter of the femur is measured with a caliper and plotted to a definite scale on coordinate paper. The exact size, shape and position of the nerves, blood vessels and lymphatics is determined; the muscles are then put in, and finally the skin with its attachments is added.

The surface of the gross specimen and the completed drawing are photographed for permanent record.

Cross section anatomy of the frog supplements dissection, and affords an exact knowledge of the relationship of the various structures, especially between the muscles and bones.

### 235. THE FRONTAL CLASPER IN THE CHIMAEROID CALLORHYNCHUS. L. Hussakof, American Museum of Natural History.

This study is based on several rare late embryos and young of the chimaeroid Callorhynchus antarcticus preserved in various collections.

The frontal clasper, present only in the male, is not formed during embryological development within the egg case, like all the other organs, including the mixopterygia. At the time of emergence from the egg case, the male specimen, with mixopterygia 1 mm., shows merely a minute, pale bulge, 1 mm. in diameter, in the median line of the head, anterior to the eyes. After hatching, this bulge gradually enlarges into an elliptical form, pointed at its anterior extremity. This continues to grow longer and wider, tapering anteriorward, and, at sexual maturity, it erupts out of the skin fully formed for functioning.

In a young fish 221 mm. long, with mixopterygia 2 mm., the frontal clasper foundation is 6 mm. long by 2 mm. at its posterior, broadest end. In a specimen 280 mm., with mixopterygia 12 mm., it is 16 mm. long, and still completely covered over by the skin of the head. In a young adult 60 cm. long, with mixopterygia 81 mm., it was out of the skin covering and evidently fully functional.

When not in use the frontal clasper in Callorhynchus folds down into the socket, anterior to its base, in which it developed, so that the frontal profile of the head is perfectly smooth, offering no restistance in swimming.

### 236. THE PROBLEM OF CYCLOMORPHOSIS IN DAPHNIA. R. E. Coker, University of North Carolina.

The paper includes a brief review of the conclusions and theories of early investigators, notably Wesenberg-Lund, Ostwald and Woltereck, respecting the origins and significance of so-called 'flotation processes' (points, 'helmets' and such), which in nature are seasonal in occurrence. A statement of the problem is offered with emphasis on the need for more precise observation and experimentation. The results of studies by the author and collaborators with a local race of Daphnia longispina are summarized. For the newborn, helmet development in the laboratory, as to presence or absence and degree of development, seems definitely subject to control by temperature, with the critical temperature in the neighborhood of 13° and the critical stage apparently about midway of larval development in the brood pouch. Nevertheless, conclusions based upon the laboratory experiments and observations of form of newborn do not hold as well as would be expected when extended to the animal in nature or to the free-living young. The complexity of the problem is discussed, with suggestions for future experimentation to resolve the apparent paradoxes. The author points out the possible interest of the problem of cyclomorphosis to students of physiology, genetics, ecology, taxonomy and evolution.

### 237. VARIATIONS IN COLOR PATTERN IN THE BLACK WIDOW SPIDER, LATRODECTUS MACTANS (FABR.). Albert M. Reese, West Virginia University.

While there is comparatively little variation in the hour-glass marking on the ventral side of the abdomen, there is a wide range of variation in the markings of the dorsal side of the abdomen.

### 238. CARTERIUS TENOSPERMA (POTTS), A SPECIES OF FRESH WATER SPONGE RECENTLY DESCRIBED AS NEW TO WISCONSIN.

James R. Niedhoefer, Marquette University. (Introduced by Paul L. Carroll.)

This specimen of Carterius tenosperma (Potts) was collected in Spider Lake, Vilas County, Wisconsin. A mass of the sponge, 15 × 5 × 3 mm., growing on the underside of the root of a tree, was found close to the shore in about 5 inches of water. This pale yellow-green sponge grew in an incrusting, elongated, irregular mass. The sponge body was rather soft and was covered by a thin film of slime. Two types of spicules projected from the body of the sponge, skeletal and dermal.

The macroscleres or skeletal spicules are tapering, long shafted, and microspined except at the tips. The microscleres or dermal spicules are short, tapering, thick shafted and profusely spined.

The gemmules are numerous and located basally. The foraminal tubule has numerous branched, twisted tendrils. The gemmule spicules or birotulates are abundantly macrospined with incurved rotules arranged in a disc around the ends of the shaft.

### 239. ADDITIONAL NORTH AMERICAN CAVE PLANARIANS.

Libbie H. Hyman, American Museum of Natural History.

Since my last report on this subject, two new cave planarians have come to light. Sorocelis americana Hyman 1938 from caves in the Ozarks of eastern Oklahoma and western Arkansas is the first finding of the Asiatic genus Sorocelis on the American continents and differs from our other cave planarians in belonging to the family Dendrocoelidae. Like the other members of the genus it has two arcs of eyes and a weak subterminal adhesive pit on the head. Sphalloplana mohri, new species, from Ezell's Cave, San Marcos, Texas, a very large broad form, reaching a length of 30 to 35 mm., is polypharyngeal, like Phagocata subterannea from Indiana caves, thus the fourth polypharyngeal planarian known in this country. It is white and eyeless and the narrowed anterior end terminates in a strong adhesive cushion, formed of the outlets of gland cells and operated by a strong muscle bundle. In its general anatomy and structure of the copulatory apparatus, Sph. mohri closely resembles its near relative, Sph. percoeca, long known from the Mammoth Caves, Kentucky. The polypharyngy of Sph. mohri again suggests derivation of all our cave planarians (except Sorocelis americana) from the epigean genus Phagocata.

### 240. NEW ANYPHAENIDAE FROM PANAMA, WITH NOTES ON KNOWN SPECIES.

A. M. Chickering, Albion College.

Anyphaenidae described and discussed in this paper were collected in the summer of 1936 during the author's third season of field work in Panama. Sixteen new species belonging to five genera (Anyphaena, Pelayo, Sillus, Teudis and Wulfila) are recognized and carefully described according to the method heretofore used by the author. Allotypes of the following known species are described: Macrophyes elongata Chickering, Wulfila modesta Chickering, W. pulverulenta Chickering. Notes on Anyphaena stylifer Cambridge, Sillus longispina Cambridge and Wulfila (Cragus) mandibulata (Petrunkevitch) are also included.

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